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Eurythoe complanata (Polychaeta: Amphinomidae), the 'cosmopolitan' fireworm, consists of at least three cryptic species

Romulo Barroso · Michelle Klautau · Antonio M. Solé-Cava · Paulo C. Paiva

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Abstract *Eurythoe complanata* (Pallas 1766) has been considered a cosmopolitan species with a great morphological similarity across its geographic range. To elucidate whether *E. complanata* is actually a single species, genetic (cytochrome oxidase subunit I and allozymes) and morphological differences were compared among specimens from the Pacific, Caribbean, and South Atlantic Oceans. Large levels of COI divergence (10–22%) and diagnostic allozyme loci identified three cryptic species: one in the eastern Pacific and two in the Atlantic, with one being morphologically differentiated and found only in islands. COI sequences between Pacific and Atlantic lineages were much more divergent than those of other transisthmian invertebrates, indicating their split before the Panama Isthmus closure or a faster evolutionary rate of COI for this species. The existence of two Atlantic species may be a consequence of parapatric speciation followed by a secondary invasion or even a sympatric speciation in the Atlantic oceanic islands.

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R. Barroso · P. C. Paiva (✉)
Instituto de Biologia, Departamento de Zoologia, Laboratório de Polychaeta, Universidade Federal do Rio de Janeiro, IB, CCS, Bl. A, Ilha do Fundão, Rio de Janeiro, RJ 21941-590, Brazil
e-mail: paulo.paiva@gmail.com

M. Klautau
Instituto de Biologia, Departamento de Zoologia, Laboratório de Biologia de Porifera, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

A. M. Solé-Cava
Instituto de Biologia, Departamento de Genética, Laboratório de Biodiversidade Molecular, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Introduction

Phenotypic distinctiveness has been the operational basis of polychaete taxonomy for a long time, primarily based on microscopic observations (Westheide and Schmidt 2003). As with many other marine taxa, this approach has led to the worldwide "lumping" of many morphologically similar, yet evolutionary distinct, species (Klautau et al. 1999; Pfenninger and Schwenk 2007), often resulting in the inflation of assumed geographic distributions (Knowlton 1993; Thorpe and Solé-Cava 1994).

In last decade, a number of molecular studies revealed several cryptic species of polychaetes within different genera, such as *Perinereis* from the English channel and the Mediterranean (Scaps et al. 2000), *Syllis* from the Mediterranean (Maltagliati et al. 2000), *Dipolydora* from the Sea of Japan (Manchenko and Radashevsky 2002), *Neanthes* and *Hediste* from the Northern Hemisphere (Breton et al. 2003), *Ophelina* from the Mediterranean (Maltagliati et al. 2004), and *Pectinaria* and *Owenia* from the Northeast Atlantic (Jolly et al. 2006). Atlantic and Pacific populations have been genetically compared for *Palola, Streblospio,* and *Polydora* (Schulze 2006; Schulze et al. 2000; Rice et al. 2008, respectively). In all cases, cryptic species were found, raising doubts on the existence of truly conspecific populations on both sides of the Americas. Generally, levels of interspecies gene divergence were much higher than those observed between conspecific populations (e.g. Schulze et al. 2000), demonstrating the taxonomic

value of molecular markers for polychaete species. This was particularly important in cases where the species had been used as pollution indicators, like in the seminal case of *Capitella capitata*, a supposedly cosmopolitan and indicator species that turned out to be, under genetic scrutiny, composed of six different biologic species (Grassle and Grassle 1976).

The concordance between distributional patterns and geological events suggests that vicariant processes and dispersal have been influential in shaping the present-day distribution of polychaete species (Glasby 2005). Thus, the origin of new taxa for polychaetes is usually regarded as a product of allopatric speciation processes. The assessment of the relative role of allopatric speciation involves the knowledge of possible barriers to gene flow as well as their efficacy (Diniz-Filho et al. 2008). Nevertheless, the identification of such barriers is difficult, due to the continuous nature of ocean waters and the presence of pelagic larval stages in most marine organisms, whose extent and direction of dispersal can be unpredictable (Palumbi 1992; Boury-Esnault et al. 1999; Lessios et al. 2003).

The amphinomid *Eurythoe complanata* presents a simple morphology, even compared to other polychaetes (e.g., absence of jaws, papillae, or teeth, morphologically very simple notopodial and neuropodial chaetae, and reduced prostomial appendages). A correlation has been indicated between the scarcity of defining morphological characteristics (i.e., species that are morphologically simple) and the breadth of apparent geographic distribution (Klautau et al. 1999). This biogeographical artifact, resulting from over-conservative taxonomy, has been termed the "low morphology problem" (van Oppen et al. 1996, but see Pfenninger and Schwenk 2007).

Eurythoe complanata was originally described by Pallas (1766) as *Aphrodita complanata*, based upon Caribbean individuals. Kinberg erected the genus *Eurythoe* in 1857, along with the descriptions of seven species. Ten years later, Kinberg (1867) expanded the number of known species of this genus through descriptions of *E. indica* from Bengal, *E. alboseta* from French Polynesia, *E. ehlersi* from Tahiti, and *E. havaica* from Hawaii. With the advance of the philosophy of taxonomical lumping during the beginning of the 20th century, the Atlantic species, *E. complanata*, was synonymized with the Pacific species *E. pacifica* (Potts 1909; Horst 1912). Subsequently, several authors (Hartman 1948; Day 1951; Ebbs 1966) followed this trend, which resulted in the lumping of about 20 previously described species from all tropical and temperate waters as junior synonyms of *E. complanata*. Thus, this species has come to be considered to present a circumtropical distribution, occurring in the Atlantic, Pacific, and Indian oceans, as well as in the Mediterranean Sea and Red Sea, inhabiting distinct microhabitats; under rocks, inside calcareous algae rhodoliths, inside corals, or even in soft sediments (Hartman 1954; Ebbs 1966; Paiva 2006).

Such broad distribution was justified based on the supposed longevity of the planktotrophic rostraria larvae of Amphinomidae (Mileikovsky 1961; Bhaud 1972) and the combination of asexual and sexual reproduction observed in this species (Kudenov 1974). However, this cosmopolitan distribution was uncertain (Kudenov 1974) based on morphological differences between larvae of "*E. complanata*" from the Pacific and the Caribbean (Marsden 1960; Kudenov 1974, respectively). Later, Kudenov (1995) suggested that other synonymies of *E. complanata* should be re-evaluated, and Barroso and Paiva (2007) questioned the cosmopolitanism of this species.

In this study, we used mitochondrial (cytochrome oxidase I (COI) sequences) and nuclear (allozymes) markers to compare levels of genetic divergence among *E. complanata* populations from both sides of the Isthmus of Panama, from the Brazilian coast and from the eastern and western Atlantic oceanic islands. Qualitative patterns of morphological variation were also investigated both among and within worldwide populations of *E. complanata*. With this approach, we specifically address the following questions: (1) Is *E. complanata* really a single, broadly distributed species? and (2) Is there any correspondence between molecular and morphological differences among largely separated populations?

Materials and methods

Sampling

Specimens of *Eurythoe complanata* were obtained from 20 sites around the world, being either collected for this study or obtained by loan from the Natural History Museum, London (BMHN) (Table 1; Fig. 1). Those used in allozyme analyses were preserved in liquid nitrogen and those for sequencing were fixed in alcohol 92%. For morphological analysis, they were anesthetized when possible in $MgCl_2$, fixed in formalin 4% and preserved in alcohol 70%. All specimens, except those used in the allozyme analyses, were deposited in polychaete collection of Federal University of Rio de Janeiro (IBUFRJ). Since the specimens from the BMHN had been fixed in formalin, they were not used for molecular analyses.

DNA amplification and sequencing

Genomic DNA was extracted using a Puregene® kit (Gentra Systems). To increase mucopolysaccharide precipitation, a final concentration of 0.4 M of SDS (sodium dodecyl sulfate) was added to the extraction buffer.

Table 1 Localities, collection dates (month/year), and number of specimens used in each analysis

On map	Locality	Date	DNA	Allozyme	Morphology	Voucher number
PAC	Taboga Island (Pacific)	08/2001	3	11	12	IBUFRJ 0543
CAR	Bocas del Toro (Caribbean)	10/2001	4	12	14	IBUFRJ 0542
CE	Ceará		–	–	4	IBUFRJ 0538
SPSP	São Pedro and São Paulo Archipelago (SPSP)	03/2005	4	–	15	IBUFRJ 0541
NOR	Fernando de Noronha Archipelago (Noronha)	03/2005	6	–	12	IBUFRJ 0547
ROC	Rocas Atoll		–	–	20	IBUFRJ 0460
PER	Pernambuco	04/2001	3	9	6	IBUFRJ 0544
ABR	Abrolhos Archipelago	04/2000	15	–	30	IBUFRJ 0540
RIO	Rio de Janeiro	08/2001	1	29	29	IBUFRJ 0537
CF	Cabo Frio	11/2002	3	25	30	IBUFRJ 0548
AZ	Azores Archipelago		–	–	6	BMNH 1955.3.1.6/10
GC	Gold Coast		–	–	44	BMNH 1953.3.1.1/17; 18/48; 49/59
AT	Atlit		–	–	2	BMNH 1937.4.7.1–5
RED	Red Sea (unknown locality)		–	–	2	BMNH 1923.3.20.8
KEN	Mombasa, Kenya		–	–	2	BMNH 1957.12.1.1/12
TAN	Oyster Bay, Tanzania		–	–	2	BMNH 1954.1.1.237/238
MOZ	Mozambique		–	–	2	BMNH 1925.1.28.122–124
SEY	Seychelles Islands		–	–	1	BMNH 82.10.16
SRI	Sri Lanka		–	–	4	BMNH 1938.5.5.7
ST	São Tomé Island	06/2007	10	–	8	IBUFRJ 0545

IBUFRJ Instituto de Biologia of the Universidade Federal do Rio de Janeiro; *BMNH* The Natural History Museum, London. Samples used for molecular and morphological analyses were collected on the same dates

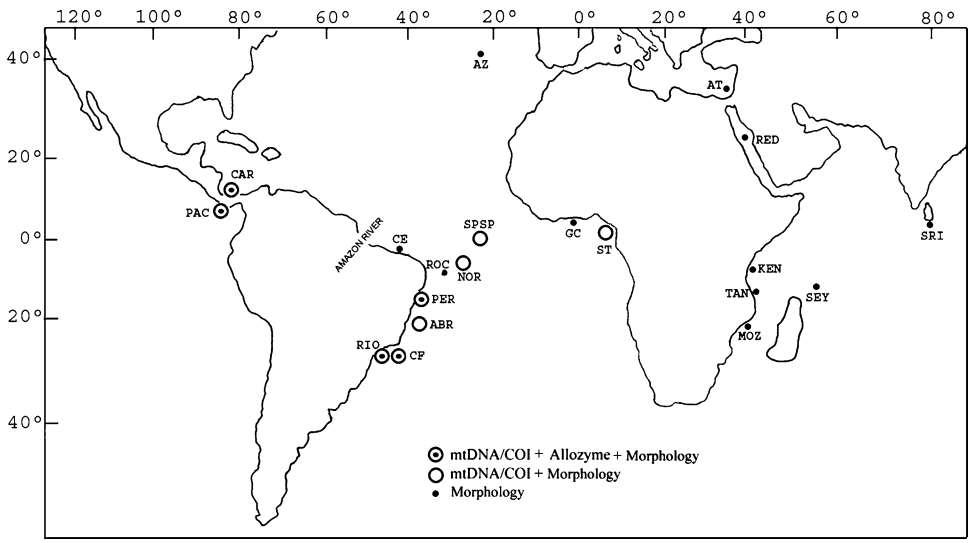

Fig. 1 *Eurythoe complanata*. Localities of the specimens analyzed. Acronyms for each locality are in Table 1

Fragments of ~600 bp of the 5′ end of the mitochondrial cytochrome oxidase I gene (COI) were amplified via polymerase chain reaction (PCR) using the universal LCO1490 and HCO2198 primers (Folmer et al. 1994). PCR reactions consisted of 1× *Taq* buffer, 2.5 mM of magnesium chloride, 0.2 mM of dNTPs, 0.6 μM of each primer, and 1 unit *Taq* DNA polymerase in a 25-μl total volume reaction. Thermal cycling conditions were 94°C for 2 min, followed by 34 cycles of 45 s at 94°C, 30 s at 49°C, and 1 min at 72°C, with a final extension step of 72°C for 3 min. PCR products were visualized by UV fluorescence on agarose gels stained with ethidium bromide, and then purified with two units each of exonuclease I and shrimp alkaline phosphatase. Sequencing was performed for both strands with fluorescently labeled dye-terminators (Applied Biosystems, Inc.). Sequences were deposited in GenBank under numbers FJ429262–FJ429280.

Molecular divergence and demographic history

Sequences were edited in the program Chromas, using the complete mitochondrial sequence of the polychaete *Platynereis dumerilli* (Audouin and Milne Edwards 1833) from GenBank (accession number NC 000931) as a guide. Edited sequences were aligned using Clustal W v1.82.

Pairwise genetic distances were estimated using Kimura's two-parameters (K2P) method (Nei and Kumar 2000) for neighbor-joining tree reconstruction and estimation of divergence time between branches. Neighbor-joining (NJ) and maximum parsimony (MP) gene trees were constructed in the program MEGA 4.0 (Tamura et al. 2007) and tested with 1,000 bootstraps. Gene trees were also inferred using maximum likelihood (ML) and Bayesian inference (BI) approaches. ML trees were inferred using the fast algorithm of Guindon and Gascuel (2003), with branch support estimated by an approximation of a likelihood ratio test (aLRT) developed within the PHYML package by Anisimova and Gascuel (2006). BI was performed using MrBayes 3.1 (Ronquist and Huelsenbeck 2003) with data partitioned by codon position (first, second, and third). Chain length (MCMC) was 10^6 generations with trees sampled every 100 generations with the first 2,500 trees being discarded as burn-in. For both BI and ML analyses, the HKY + Γ model of molecular evolution was used. The model and parameters for the analyses were determined with Modeltest 2.2 (Nylander 2004), according to the hierarchical likelihood ratio test criterion (hLRT) (Posada and Crandall 1998).

Gene trees were rooted using a sequence of the amphinomid *Hermodice carunculata* (Pallas 1766), which was collected in northeast Brazil. The possibility of saturation in the rate of base substitutions at the COI gene was assessed by the methods of Xia et al. (2003) using the DAMBE program (Xia and Xie 2001). A relative-rate test (Takezaki et al. 1995) was performed with the PHYLTEST program (Kumar 1996) to compare divergence rates across the Kimura two-parameters gene tree, to verify if the molecular clock hypothesis would not be applicable to our data. The program DnaSP version 4 (Rozas et al. 2003) was employed to test the neutrality of nucleotide substitutions or possible historical population size changes, using Tajima's *D*, Fu's *F*s, and Ramos-Onsins with Rozas' R_2 tests (Tajima 1989; Nei and Kumar 2000; Ramos-Onsins and Rozas 2002). Confidence intervals for each statistic were estimated by means of computer simulations using the coalescence algorithm. An analysis of molecular variance (AMOVA) was performed using the program Arlequin 3.0 (Excoffier et al. 2005). A Mantel test was performed to assess the relationship between molecular divergence (mtDNA; K2P distances) and geographic distance using *Z* statistics (Sokal and Rholf 1995).

Allozymes

Horizontal 13% starch gel electrophoresis was performed using standard methods (Solé-Cava and Thorpe 1986; Murphy et al. 1990). The buffer system used was the Tris-EDTA-Maleate pH 7.4 (Hillis et al. 1996).

Of the 19 enzyme systems assayed, only six met the stringent conditions of clear resolution and reproducibility: catalase (*Cat*, Enzyme Commission Number, E.C. 1.11.1.6), phosphoglucomutase (*Pgm*, E.C. 5.4.2.2), malate dehydrogenase (*Mdh*, E.C. 1.1.1.37), phosphogluconate dehydrogenase (*Pgd*, E.C. 1.1.1.44), isocitrate dehydrogenase (*Idh*, E.C. 1.1.1.42), and mannose phosphate isomerase (*Mpi*, E.C. 5.3.1.8). Enzyme staining followed the methods of Manchenko (1994).

Genotype frequency data were used to calculate gene frequencies and fit to Hardy–Weinberg equilibrium. Unweighted pair-group mean analysis (UPGMA, Sneath and Sokal 1973) was utilized to represent genetic relationships between populations using unbiased pairwise gene identities (Nei 1978). This method is less biased by the large stochastic errors associated with estimating gene identities over small numbers of loci (Nei 1987). All calculations were performed using the TFPGA 1.3 program (Miller 1997).

Morphological analyses

Morphological analysis was performed through observation of the diagnostic traits normally used in the taxonomy of Amphinomidae species (Kudenov 1995): prostomium and prostomial appendage morphology and relative position, eyes, caruncle, morphology and distribution of both notochaeta and neurochaeta (sampled in anterior, medium, and posterior chaetigers), shape, as well as location and distribution, of branchiae and cirri (notopodial and neuropodial).

For chaetal ultrastructure analysis, specimens were critical point dried, covered with 25 nm of gold and examined under scanning electron microscope (SEM) at the National Museum of Federal University of Rio de Janeiro. For measurement of oocyte sizes, fixed worms were ripped up with fine metal forceps. The diameters of oocytes were measured using a compound microscope with an ocular micrometer.

Results

Sequence analyses

All tree topologies (NJ, MP, ML, and BI) were congruent regarding both the split of Pacific/Atlantic lineages and the divergence of the Atlantic clade into two subclades (Fig. 2). The divergence between sequences from eastern Pacific

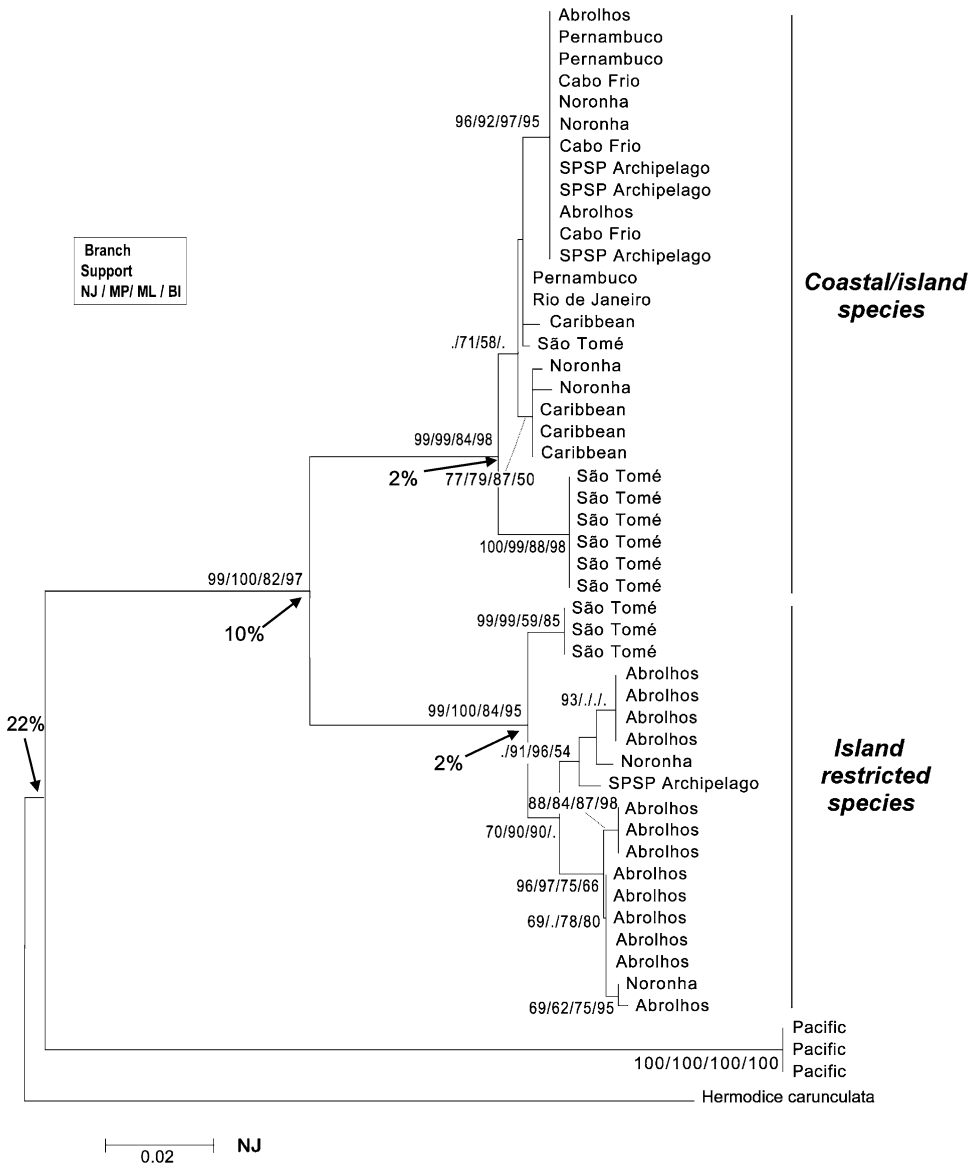

Fig. 2 Neighbor-joining (*NJ*) tree of *E.* cf. *complanata* specimens from eastern Pacific, Caribbean Sea and South Atlantic Ocean. Branch supports (>50%) presented are from (*NJ*), maximum parsimony (*MP*), maximum likelihood (*ML*) and Bayesian inference (*BI*). The tree is drawn to scale, with branch lengths in units of number of base substitutions per site computed using the Kimura two-parameters method. Branch support = bootstrap test (1,000 replicates) or posterior probability (*BI*)

Table 2 Population parameters for each putative Atlantic species (acronyms of localities according to Fig. 1)

Putative species	*N*	*H*	π (SE)	*D*	*Fs*	R_2
Coastal/Island (CAR, SPSP, NOR, PER, ABR, RIO, CF, ST)	26	11	0.010 (0.005)	0.51 (0.74)	0.11 (0.54)	0.14 (0.66)
Island-restricted (SPSP, NOR, ABR, ST)	19	12	0.011 (0.006)	−0.32 (0.44)	−1.86 (0.21)	0.12 (0.38)

N = number of sequences; *H* = number of haplotypes; π = nucleotide diversity (with standard error); *D* = Tajima's test; *Fs* = Fu's statistic; R_2 = Ramos-Onsins & Rozas statistic. For tests of neutrality and population changes probabilities are provided within brackets

(Panama) and Atlantic samples was 22%, representing 111–116 nucleotide differences. The Atlantic samples were further separated into two subclades, which diverged 10% from each other, representing 48–58 nucleotide differences. The most recent cladogenic event seems to be the split between the two Atlantic species, and the most ancient was the split between the Pacific and Atlantic lineages.

Substitutions within the *Eurythoe complanata* clades were all silent with an average transition/transversion ratio of 15.15 (with gamma correction). No evidence of saturation in nucleotide substitutions was observed (Xia test; $P < 0.001$). The neutral model of nucleotide substitutions could not be rejected for the sequences of any of the analyzed species (Table 2).

Biogeographical patterns of the highly divergent (10% K2P) Atlantic clades can be summarized as follows: (1) one clade was composed of specimens occurring along the coast from the Caribbean Sea (Panama) to southern Brazil

Table 3 Results of the analysis of molecular variance (AMOVA) of COI sequence data of the coastal island species (Caribbean × Brazil × Africa) and island-restricted species (Brazil × Africa)

Source of variation	*df*	Sum of squares	Variance	% Variation
Coastal/Island species				
Among groups	2	29.643	1.758	61.17
Among localities (within groups)	5	6.438	0.107	3.72
Within populations	19	19.179	1.009	35.11
Total	26	55.259	2.874	
Island-restricted species				
Among groups	1	21.266	2.849	46.60
Among localities (within groups)	2	10.005	1.111	18.18
Within populations	15	32.308	2.154	35.22
Total	18	63.579	6.114	

and also included the western and eastern Atlantic islands (this clade is referred to as the *coastal/island species*) and (2) one clade composed of specimens found only on the east and west Atlantic islands (São Pedro and São Paulo Archipelago, São Tomé, Fernando de Noronha Archipelago, and Abrolhos Archipelago; this clade is referred to as the *island-restricted species*). Population parameters were estimated separately for each species (Table 2).

High levels of population structure were observed within each putative species in an analysis of molecular variance, AMOVA (Table 3). The AMOVA approach was applied for haplotypes within localities grouped in three populations: the Brazilian coast, the Caribbean, and Africa. Significant differences were detected between Brazilian ($n = 16$) and African ($n = 3$) populations of the island-restricted clade species ($\Phi_{ST} = 0.64$, $P < 0.001$). For the coastal/island clade species, significant differences were detected among Caribbean ($n = 4$), Brazilian ($n = 16$), and African ($n = 7$) populations ($\Phi_{ST} = 0.65$, $P < 0.001$), as well as between populations from Africa and western Atlantic (Brazil + Caribbean pooled); although these differences were of a lesser extent, they were still significant ($\Phi_{ST} = 0.41$, $P < 0.001$). For the coastal/island clade species, pairwise comparisons of the three populations yielded Φ_{ST} values of 0.48 (Brazilian × Caribbean), 0.76 (African × Caribbean), and 0.80 (African × Brazil), which were all highly significant ($P < 0.001$). Thus, measured population parameters showed that both species are widely distributed and present a large intraspecific population structure. A Mantel test demonstrated a significant and positive correlation between geographic distance and molecular divergence for the coastal/island species ($P < 0.001$). This correlation was still significant ($P < 0.038$) when African samples ($n = 7$) were removed. Nevertheless, there was no correlation ($P < 0.86$) when Caribbean specimens were excluded from the analysis. This indicates a lack of isolation by distance along the 2,500 km of Brazilian coast. Owing to the small number of African samples ($n = 3$), the test was not applied for the island-restricted species.

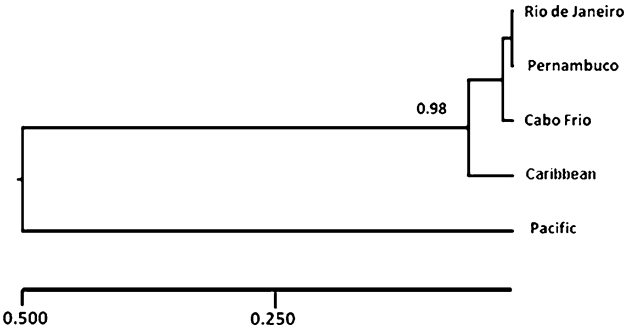

Fig. 3 UPGMA tree of Atlantic and Pacific populations of *Eurythoe* cf. *complanata*, based on allozyme unbiased genetic distances (Nei 1978)

The molecular clock hypothesis was not rejected in constancy rate tests (Takezaki et al. 1995) in the comparison between Atlantic and Pacific lineages ($Z = 1.88$; $P > 0.05$) or between the two Atlantic lineages ($Z = 0.04$; $P > 0.05$) using *Hermodice carunculata* as outgroup.

Allozymes

Two loci (*PGM* and *MPI*), out of the six loci analyzed, presented exclusive alleles in the Atlantic and Pacific coasts of Panama, although only *MPI* was diagnostic of each group. The Atlantic and the Pacific populations could also be clearly separated based on high genetic distances between them (Fig. 3, S1 in ESM). In contrast, the levels of gene divergence between the Caribbean and southwest Atlantic populations were comparable to those typically observed between conspecific populations (Thorpe and Solé-Cava 1994).

Morphology

After a careful examination of 247 individuals from 20 different localities, no morphological feature could be used to distinguish samples from different localities. Instead, a large intrapopulational variation was observed for some

characteristics, such as the shape of bifurcate notochaetae and their distribution along the body, the extension of the caruncle, the position of median antennae on prostomium (aligned to the first or second pair of eyes), and patterns of eye pigmentation. A noticeable pattern was the variation of bifurcate notochaetae along the body, with the longer prong increasing in size in posterior chaetigers, whereas the smaller prong decreased and was practically absent from chaetiger 7–9 onward, remaining only as a spur. Although the bifurcate notochaetae were typically smooth, some individuals presented denticulations on the inner side of the longer prong of the chaetae (S2 in ESM). In contrast to the notochaetae, neurochaetae did not present any noticeable variations along the body. This was also noted for the caruncle, which reached the third chaetiger in almost all specimens analyzed. Exceptions in this pattern were seven specimens from the Gold Coast (Africa), two from Seychelles Island and one from Mozambique (Africa), with caruncles reaching the second chaetiger, and one specimen from the Gold Coast (Africa), one from the Red Sea, and one from the Indian Ocean (Mombasa), whose caruncles reached the fourth chaetiger.

Interestingly, when the chaetae of specimens from the three main clades identified by COI molecular analysis were analyzed separately by SEM, the Atlantic island-restricted clade could be distinguished from the other species, including its sympatric counterpart (the Atlantic coastal/island species), by the absence of harpoon notochaetae (Fig. 4).

Despite the high number of specimens and populations observed, only four ripe females were found, all of them from the Caribbean (collected in October/2001). Mean diameter (M) and standard error (SE) of the oocytes for each female were $M = 83.8$ µm (SE = 6.0; $n = 20$); $M = 81.2$ µm (SE = 2.6; $n = 6$); $M = 81.2$ µm (SE = 2.3; $n = 8$); and $M = 81$ µm (SE = 3.3; $n = 10$). Overall, mean of oocyte diameter was 82.3 µm (SE = 4.7; $n = 44$).

Discussion

The genetic analyses clearly indicate the existence of three cryptic species within *Eurythoe complanata*, showing that the previously assumed cosmopolitanism of this species is a taxonomic artifact. The east Pacific and Atlantic samples clustered in deeply divergent (mtDNA: Kimura two-parameters; K2P = 22%; allozymes: genetic distance, $D = 0.50$) reciprocally monophyletic clades. Within the Atlantic Ocean, we were able to further identify two cryptic species, which differed in their COI sequences (mtDNA: K2P = 0.10) and formed reciprocally monophyletic clades: (1) a 'coastal/island species' distributed from the Caribbean Sea (*locus typicus*) to southern Brazil (Rio de Janeiro), including the Brazilian oceanic islands and the African island of São Tomé; and (2) a species living in sympatry with the coastal/island species only in the Atlantic islands (Brazil and Africa), referred to as the 'island-restricted species'.

Levels of COI gene divergence (K2P distance = 0.22) between Pacific and Atlantic species of *Eurythoe* were in the same range as those observed between other congeneric polychaete species like *Streblospio* spp. (0.19) (Schulze et al. 2000), *Palola* spp. (0.24) (Schulze 2006), and *Chaetopterus* spp. (0.24) (Osborn et al. 2007). Gene divergence between the Atlantic lineages of *Eurythoe* was smaller (K2P = 0.10), but similar to those observed for cryptic species of *Pectinaria koreni* (0.16) (Joly et al. 2005) and close related species of *Paranaitis* (0.14) (Nygren et al. 2009).

Molecular divergences in mtDNA sequences between Pacific and Atlantic *Eurythoe* species were higher than those usually observed between geminate pairs of other transisthmian invertebrates (Knowlton and Weigt 1998; Lessios et al. 2003). Such deep divergence may indicate that the species compared are not geminate pairs, and that the divergence between those species occurred well before the closure of the isthmus (see e.g. Marko 2002; Lessios 2008 for a discussion). Alternatively, these species may comprise an actual geminate pair, and the high divergence observed between COI sequences has resulted from a rate of molecular evolution for that gene in *Eurythoe* that is at least three times higher than that observed in other invertebrate species. The two hypotheses differ in the relative importance given to the effective mutation rate (μ) and the time since the splitting of the two lineages (τ) and are very hard to discriminate without knowledge on historical population sizes of each species (Hickerson et al. 2003). Discussing the molecular clock and its calibration is beyond the scope of this work, but, in any case, it remains clear that the Pacific and Atlantic species of *E.* cf. *complanata* are genetically very divergent and warrant specific status.

The high dispersal power of *E. complanata*, as expected for a polychaete with planktotrophic teleplanic larvae (Bhaud 1972; Kudenov 1974), is confirmed by the occurrence of conspecific populations along the Caribbean and Brazilian coast, including the oceanic islands from both sides of the Atlantic Ocean (Brazilian and African islands). The existence of a different species in the South Atlantic islands may be the result of ecological/sympatric speciation during island formation or an allopatric/parapatric speciation event with subsequent secondary contact.

A sympatric or parapatric speciation event could be related to an adaptive divergence to a new environment (Rundle and Nosil 2005), represented by the shallow bottoms that appeared during the formation of the southern Atlantic islands. Rocha et al. (2005) suggested that ecological speciation may explain why some Caribbean reef fishes

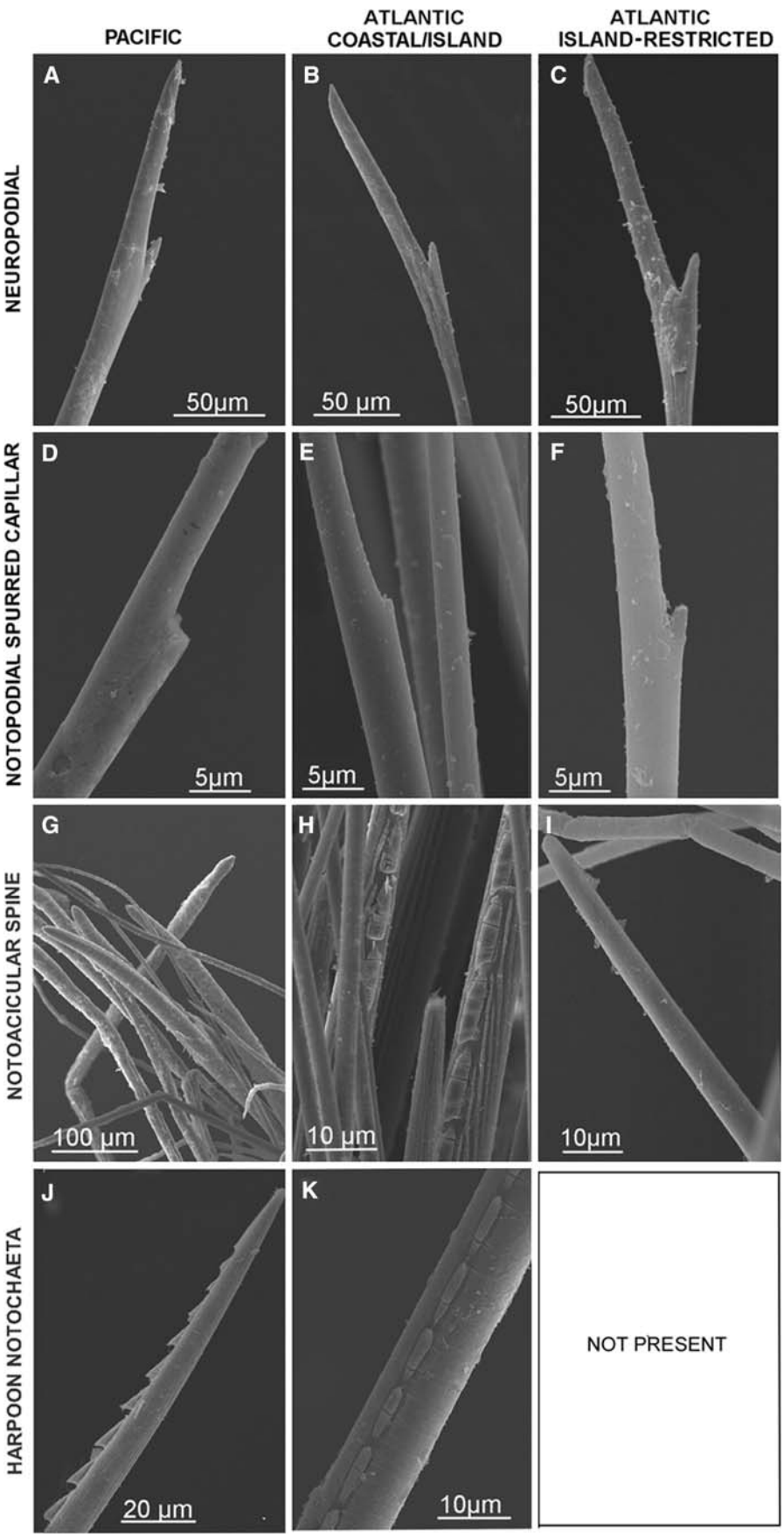

Fig. 4 Chaetal types of the three genetically differentiated species of *Eurythoe*. **a**–**c** Neuropodial chaeta, **d**–**f** Notopodial spured capillar chaeta, **g**–**i** Notoacicular spines, **j**, **k** Harpoon notochaeta, **a**, **d**, **g**, **j** Pacific species, **b**, **e**, **h**, **k** Atlantic coastal/island species, **c**, **f**, **i** Atlantic island-restricted species

do not exist on the Brazilian coast, but are common in the Brazilian oceanic islands. However, the origin of southern Atlantic oceanic islands (ca. 100–9 Ma) clearly predates the splitting time of the two Atlantic clades of *Eurythoe* (ca. 1.4 Ma, if we accept the hypothesis of a fast COI clock of 7% divergence/Ma in the genus, based on the transisthmian pair analyzed, or 5 Ma, in the Pliocene, using the coalescent calibration clock of 2% COI divergence/Ma of Hickerson et al. 2003). The lower estimated divergence time corresponds to the Pleistocene glaciation, which was a period of intense speciation and radiation events, mainly for nearshore marine forms presently connected but isolated in the past (Palumbi 1994). Lower sea levels between 1.6 and 1.4 Ma may have resulted in several pulses of marine speciation and extinction, mainly in Caribbean waters (O'Hara and Poore 2000; Dawson 2005), and were considered responsible for the separation of extant sympatric species of the sea-urchin genus *Echinometra* in the Atlantic (McCartney et al. 2000). This scenario would be compatible with an allopatric or, more likely, parapatric speciation (Coyne and Orr 2004; Rocha and Bowen 2008) of the two species of *Eurythoe*, because these species inhabit a fragmented environment (reefs or rocky shores) and include pelagic larvae in their life cycles.

Genetic connectivity between populations from both sides of the South Atlantic, such as that observed in the two species of *Eurythoe*, has also been found in other marine animals, including sea urchins (Lessios et al. 1998; McCartney et al. 2000) and reef fishes (Bowen et al. 2006; Floeter et al. 2008). One possible explanation for this connectivity could be the transport of the teleplanic larvae by the westward South Equatorial Current and the eastward South Equatorial Countercurrent (Brown 1990). Another possibility is the anthropogenic transport of larvae by ship ballast water (Bastrop and Blank 2006; Blank et al. 2007) or of adults through hull fouling (Farrapeira et al. 2007).

The conspecific Caribbean and Brazilian populations of coastal/island species seem to present a moderate degree of differentiation ($\Phi_{ST} = 0.48$; $P < 0.001$). This indicates that the Amazon River may act as a barrier restricting gene flow between populations on either side. The Amazon River seems to be a barrier to gene flow for several invertebrates, such as lobsters (Sarver et al. 1998) and some sea urchins (Lessios et al. 2003), but not for other species, such as sponges (Lazoski et al. 2001), ascidians (Nóbrega et al. 2004), and sea urchins (Zigler and Lessios 2004). Reef and intertidal fishes exemplify the contrasting role of the Amazon River plume as a biogeographical barrier for marine species between Brazil and Caribbean region (Rocha 2003; Lima et al. 2005). Thus, both the deep waters of the Atlantic Ocean and the outflow of the Amazon River are likely to act as filters separating populations, including the two cryptic Atlantic species of *Eurythoe*.

Despite the higher molecular divergence, species of *Eurythoe* cf. *complanata* from the Pacific and Atlantic coastal/island are morphologically similar, while the Atlantic island-restricted species can be differentiated from these two other species by the absence of harpoon notochaetae (Fig. 4). Specimens of *E. complanata* from the Atlantic and Pacific were considered morphologically similar by Kudenov (1974). The individuals examined here correspond to the detailed descriptions of McIntosh (1885) and Chamberlin (1919) for the Pacific Ocean, and of Ebbs (1966) for the Atlantic coastal/island. Even though we could not find differences in oocyte size between such divergent species, we cannot exclude the possibility that differences may exist in other developmental modes characters as observed for cryptic species of *Scoloplos armiger* living in sympatry in the North Sea (Kruse and Reise 2003).

Differences among specimens, such as the denticulations on the longer prongs of bifurcate notochaetae, have already been referred to in other studies (McIntosh 1885; Hartman 1940; Day 1951; Nunez et al. 1991), as well as the denticulation in bifurcate neurochaeta (Barroso and Paiva 2007). Ebbs (1966) suggested that denticulations in chaetae are the product of erosion, whereas Day (1951) argued that *E. complanata* chaetae could be easily modified through preservation methods. The analysis of several specimens of many different localities suggests that the presence of denticulations on some chaetae, as previously discussed, is indeed due to an intraspecific variation of unknown origin (i.e., erosion, artifact of preservation methods, or intrapopulation variability), and, hence, not useful for species delimitation.

The oogenesis process in *E.* cf. *complanata* was reported to occur from May to August in the eastern Pacific (Kudenov 1974), i.e. 2 months before of the occurrence of ripe females in the Caribbean. Notwithstanding that difference, the mean oocyte diameter recorded here for the Caribbean specimens was similar to that reported for the Pacific (82.3 μm, this study; 87.5 μm, Kudenov 1974).

Eurythoe complanata was originally described based on Caribbean specimens, but specific types are missing, and the precise type locality is unknown. This means that either of the two Atlantic species could correspond to *E. complanata* since the description may have been based on coastal/island or island-restricted specimens. Considering that all analyzed Caribbean specimens were included in the coastal/island clade, we decided to assign the coastal/island clade to *E. complanata*. The Atlantic island-restricted species is probably *Eurythoe laevisetis* described by Fauvel (1914) and differentiated from *E. complanata* by the lack of harpoon notochaetae. Furthermore, the type locality of *E. laevisetis* is São Tomé Island, one of the sites where we found both species in sympatry. This species has been considered a junior synonym of *E. complanata* by several authors (e.g. Fauvel 1947; Ebbs 1966), but we believe that

conclusion to be wrong and suggest that the species name *E. laevisetis* be used for the island-restricted *Eurythoe* similar to *E. complanata* but without harpoon notochaetae.

The species from the Pacific coast of Panama, clearly differentiated by molecular analysis from *E. complanata*, should be denominated *Eurythoe armata* (Kinberg 1867), a species described from the Pacific coast of Panama as *Blenda armata* but later considered a junior synonym of *Eurythoe complanata* by Hartman (1948). Kinberg (1857) described three other species of the genus *Eurythoe* from the Pacific Ocean (*E. corallina* and *E. kamechamecha* from Hawaii, and *E. pacifica* from French Polynesia). Those species would have priority over *Blenda armata*, but it may be inappropriate to refer to species from the western Pacific to the same species from the Panamanian Coast, given that the Pacific Ocean is considered a deep dispersal barrier between such distant localities (Lessios et al. 2003).

Given its ubiquity and putative cosmopolitanism, "*E. complanata*" has been used as an indicator of heavy metal contamination in Mexico (Vázquez-Núñez et al. 2007) and California (Mendez and Paez-Osuna 1998), and as a source of bioactive compounds in Japan (Nakamura et al. 2008). The discovery of three cryptic species of *Eurythoe* within the Atlantic and the east Pacific advises against the extrapolation of the results obtained with samples from one geographic area to others, and raises strong doubts on the supposed occurrence of *E. complanata* outside the Atlantic Ocean.

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