SHORT COMMUNICATION

Genetic variability of Acartia tonsa (Crustacea: Copepoda) on the Brazilian coast

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We determined the genetic structure of Acartia tonsa using sequences of the mtCOI gene obtained from 58 specimens collected on the Brazilian coast. Variations in the sequences discriminated three distinct lineages, with genetic distances of 6–16%. The genetic structuring observed in the Brazilian A. tonsa populations indicates that they are heterogeneous.

KEYWORDS: mtDNA; zooplankton; genetic structure

Marine plankton is characterized by a large number of populations and their potential for dispersal by ocean currents, although it is unclear whether they may represent the same species or a number of different cryptic species. A number of studies have detected genetic structuring over a range of spatial scales, including ocean basins, gyres within the same ocean basin and, regionally, among coastal features such as estuaries and inlets (Goetze, 2003; Blanco-Bercial et al., 2011; Chen and Hare, 2011). Patterns of genetic structuring may also vary between holoplanktonic species from the same site (e.g. Eucalanus hyalinus vs. Eucalanus spinifer; Goetze, 2005). The calanoid copepod Acartia tonsa, a species described more than 150 years ago, is seasonally dominant in many coastal and estuarine environments in the Atlantic and Indo-Pacific (Mauchline, 1998). Recent genetic analyses

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have indicated that genetic structuring may be prevalent and complex in A. tonsa populations (Caudill and Bucklin, 2004; Chen and Hare, 2008, 2011; Costa et al., 2011).

Despite the geographic distribution of A. tonsa, its euryhaline and eurythermal characteristics and also marked genetic diversity (Caudill and Bucklin, 2004; Chen and Hare, 2008, 2011; Costa et al., 2011), few data are available on the species from some regions, in particular the Southern Hemisphere. In the present study, mitochondrial DNA sequences of A. tonsa from a number of coastal Brazilian sites were analyzed in order to determine whether they represent geographically distinct genetic populations.

Copepods were collected from five distinct Brazilian coastal systems, in the states of Amapá, Pará, Maranhão, Bahia and Paraná (Fig. 2). The samples were collected by horizontal trawls of the subsurface water with a conical–cylindrical plankton net (125-μm mesh size), and preserved immediately in 70% ethanol. In the laboratory, all the specimens of A. tonsa were separated and identified (Bradford-Grieve et al., 1999). DNA was extracted from 58 individuals, 11 from the coast of Amapá (AP), 13 from the Paracatu River (PA), 15 from São Marcos Bay (MA), 9 from Graçã beach (BA) and 10 from Pontal do Sul beach (PR), using a Wizard Genomic Purification kit. A 553-bp region of the mitochondrial cytochrome oxidase I gene was amplified using the primers COI_VH and L1384 (Folmer et al., 1994). The amplification and sequencing protocols were the same as those used by Costa et al. (Costa et al., 2011). The sequences obtained here were registered at GenBank under accession numbers KM458075-87. The genetic distances (p distances) between the lineages were defined using MEGA version 5.2.2 (Tamura et al., 2011). The phylogenetic relationships among the A. tonsa populations were evaluated using PhyML 3.0 (Guindon et al., 2009). A haplotype network was constructed in Haploviewer (Salzbürger et al., 2011) and indices of genetic diversity (h) were calculated in ARLEQUIN (Excoffier and Lischer, 2010).

A total of 13 distinct haplotypes were identified, based on 109 variable sites, representing three lineages (L1, L2 and L3), which appear to represent a complex of cryptic species, as proposed in previous studies of specimens from the north of Brazil (Costa et al., 2011) and the east coast of the USA (Caudill and Bucklin, 2004; Chen and Hare, 2008, 2011). The L1, L2 and L3 lineages diverge by 6–16%. The levels of divergence between L1–L2 (15%), and L1–L3 (16%) and L2–L3 (6%) are consistent with the presence of at least three cryptic species of A. tonsa, with L2 being closer to the L3 lineage (Fig. 1). Figure 1 demonstrates that differences

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**Fig. 1.** Phylogeny of the A. tonsa lineages. The numbers adjacent to the nodes are bootstrap values derived from the maximum-likelihood method. The congeners, A. hudsonica (EU274431), A. danae (EU856796), and A. negligens (EU856812) were used as the outgroup. Two sequences of A. tonsa (HQ661802-03) were used in phylogenetic analyses.
among species are more enhanced than those found in the *A. tonsa* populations.

The divergence found between these lineages is consistent with that observed between distinct species or even genera of other planktonic calanoid copepods (see Bucklin et al., 2003). However, comparisons with the outgroup indicate that the *A. tonsa* lineages have diverged relatively recently in comparison with other *Acartia* species (Fig. 1). The genetic distances recorded in the present study are similar to those reported by Chen and Hare (Chen and Hare, 2011), who distinguished at least three distinct clades of 11.4–14.2%. The results of the present study thus indicate that the levels of genetic distance found were consistent with the existence of distinct lineages.

In L1, most of the individuals presented two common haplotypes and/or one of the unique haplotypes. The specimens collected in Amapá and Pará all belonged to the L1 lineage, indicating possible regional structuring. This lineage was described previously by Costa et al. (Costa et al., 2011). The haplotype network (Fig. 2) is characterized by the extensive overlap of haplotypes from the L1 lineage with those of other sites except Bahia, as well as a reduced diversity among these haplotypes ($h = 0.6000 \pm 0.1305$). Moderate and low diversities were also observed in L2 ($h = 0.7857 \pm 0.0587$) and L3 ($h = 0.4530 \pm 0.0869$), respectively.

The haplotype network shows that all three *A. tonsa* lineages occur on the coast of Paraná, sharing haplotypes with other populations, as well as presenting unique haplotypes, indicating that despite the considerable distance between the populations in Paraná and the other Brazilian states, gene flow occurs between them. The overlap between lineages does not appear to be related to the role of ocean currents as dispersal mechanisms, given that specimens from Paraná shared haplotypes with all the other populations. This contradicts the idea of geographic structuring, although specimens from the northernmost states of Amapá and Pará were included only in L1.

The gene flow between northern and southern Brazil reduces the genetic divergence between local populations. This overlap contradicts the hypothesis of dispersal mechanisms, and more conclusive evidence on the possible occurrence of transfer in ship ballast water is needed. The presence of three different lineages in Paraná may in fact reflect distinct adaptations to factors such as seasonal variation in the water temperature and salinity (see Marengo, 1995; Nittrouer and DeMaster, 1996).

Overall, the genetic divergence found here distinguished three *A. tonsa* lineages, which are well-defined genetically. This emphasizes the need for the analysis of additional molecular markers from a much wider geographical area and time scale, as well as more detailed studies of the morphological variation in the cryptic *A. tonsa* species.

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