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Worms without borders: genetic diversity patterns in four Brazilian *Ototyphlonemertes* species (Nemertea, Hoplonemertea)

Sónia C. S. Andrade · Jon L. Norenburg · Vera N. Solferini

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Abstract Understanding the evolutionary processes from recent demographic history is especially difficult for interstitial organisms due to their poorly known natural history. In this study, the genetic variation and population history of the four *Ototyphlonemertes* (Diesing in Sitz ber Math Nat Kl Akad Wiss Wien 46:413–416, 1863) species were evaluated from samples collected along the Brazilian coast (between 27°31′S and 13°00′W) in 2006. The mitochondrial region cytochrome *c* oxidase subunit 3 (COX3) is analyzed to assess the genetic variation of these dioecious species. Although these species have a sympatric distribution along the coast, our data suggest that their levels of differentiation and their demographic histories differ sharply. There is strong evidence of gene flow among demes in *O. erneba* and *O. evelinae*, and their level of structuring is much lower than for the other two species. Indeed, the COX3 fragment reveals cryptic lineages in *O. lactea* and *O. parmula*. The results seem to contradict the high genetic structuring and low intrapopulational variability expected with the ecological constriction and habitat discontinuity faced by these organisms, meaning that there might be gene flow among populations or their dispersal capability has been underestimated.

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S. C. S. Andrade · J. L. Norenburg
Department of Invertebrate Zoology,
National Museum of Natural History,
Washington, DC 20560-0163, USA

S. C. S. Andrade · V. N. Solferini
Departamento de Genética, Evolução e Bioagentes,
Universidade Estadual de Campinas, Campinas,
SP 13083-970, Brazil

S. C. S. Andrade (✉)
Museum of Comparative Zoology,
Department of Organismic and Evolutionary Biology,
Harvard University, 26 Oxford St, Cambridge, MA 02138, USA
e-mail: sandrade@fas.harvard.edu

Introduction

The magnitude of populational connectivity, its spatial scale, and its determining factors comprise a central question in population genetics. For adjacent coastal marine populations, the physical and ecological forces that promote genetic differentiation, as well as the processes involved in maintaining it, still are poorly understood (Dawson 2001; Selkoe et al. 2010). In this regard, comparative phylogeography has been used as a tool to identify historical factors that have shaped the histories of co-distributed species (e.g., Avise et al. 1987; Rocha et al. 2002, 2008; Abellán et al. 2009).

Concordant genealogical patterns for several species are likely to represent shared historical demography, whereas discordant patterns may help elucidate (1) different responses to geographic barriers, selective gradients, and historical events and (2) the role of bionomic traits (Avise 2000). In non-model organisms, attaining statistical power to distinguish the effects of demographic history from the remaining factors is rather difficult. In spite of that, population genetic and phylogeographic studies often provide the only means to infer the recent demographic history of natural populations occupying poorly known environments for which sample sizes often are restricted. Marine invertebrates that inhabit permanently the interstitial sand environment are uniquely constrained by the environment, such that they can be characterized not only as meiofauna (defined by size alone) but as a specialized biocenosis, the

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interstitial fauna or mesopsammon (Higgins and Thiel 1988). Disjunct distributions, often including patches of less than a few square meters, are characteristic of this type of fauna. Most of the species that comprise the so-called meiofauna lack distinctive free-swimming larval stages, and the adults are not likely to swim actively in the open water (Westheide et al. 2003). Thus, innate dispersal abilities are considered low for meiofaunal taxa, and high structuring among populations and low intrapopulational variability are expected (e.g., Rocha-Olivares et al. 2001; Casu and Curini-Galletti 2004, 2006; Derycke et al. 2008). However, entirely unknown is the contribution to dispersal by adult meiofauna entrained in the water column, but it may represent a significant factor for some taxa (e.g., Shanks and Walters 1997), and occasional rafting of adults in ship ballast could be locally significant.

Ototyphlonemertes (Hoplonemertea) is a widespread and morphologically distinctive genus of small and slender nemerteans. Its members mostly are found in the pore interstices of discontinuously distributed patches of coarse-grained, shallow marine sediments from the upper to subtidal zone (Envall 1996). *Ototyphlonemertes*, with 22 recognized species (Envall and Norenburg 2001; Chernyshev 2007), is relatively species-rich among nemertean genera, but unambiguous morphological diagnoses for many are problematic, either for poor data or when encompassing variability. Therefore, Envall and Norenburg (2001) established six "phylomorphs," small and morphologically homogeneous groups of morphotypes—Duplex, Pallida, Cirrula, Fila, Lactea, and Macintoshi—that could be diagnosed unambiguously, although one or more may be paraphyletic or even polyphyletic.

Meiofauna typically are characterized as having direct development and lacking free-swimming larvae (Giere 2009), but a planktonic lecithotrophic larva is known for several species of the genus *Ototyphlonemertes* (Chernyshev 2000; Norenburg and Stricker 2002), thus implying potential—if limited—migration capability. The species can be considered a "closed system," characterized by local larval retention and susceptible to recurrent extinctions and re-colonizations (Cowen et al. 2000; Heads 2005; Derycke et al. 2008). The lack of gene flow due to the larval retention in "closed systems" should allow allopatric differentiation, and potentially speciation. *Ototyphlonemertes* species also present morphological clines (Envall and Norenburg 2001), potential evidence of rapid divergence following local adaptation. This pattern should be consistent with the model of recurring adaptive radiations taking place at small geographic scales in allopatrically distributed sister taxa. Delimiting species boundaries in marine taxa is a challenging task (Knowlton 1993, 2000; Hart and Sunday 2007), and this is especially true for nemerteans, due to the scarcity of discrete morphological traits. Several of the traits used to distinguish varieties among established species are plastic and would rather diagnose ecological morphs than represent monophyletic units (Envall and Sundberg 1998; Envall and Norenburg 2001). The lack of correspondence between morphology and evolutionary lineages in several genera of nemerteans could result from the presence of multiple sibling species (Envall and Sundberg 1998; Sundberg et al. 2009; Chen et al. 2010). In recent years, the number of molecular studies addressing the cryptic diversity in nemerteans has increased substantially (e.g., Strand and Sundberg 2005a; Mateos and Giribet 2008; Thornhill et al. 2008; Sundberg et al. 2009; Chen et al. 2010; Mahon et al. 2010).

Few molecular studies have been conducted along the 8,000 km of the Brazilian coast, which includes diverse habitats. Populational genetics or phylogeographic studies from meiofauna species are still underrepresented when considering the large diversity in the Brazilian coast (Winston and Migotto 2005; Albuquerque et al. 2007; Di Domenico et al. 2009; Venekey et al. 2010). In this study, we analyzed patterns of genetic diversity based on mtDNA sequences in four widespread *Ototyphlonemertes* species collected along the Brazilian coast: *O. erneba* Corrêa (1950) (Pallida morph), *O. evelinae* Corrêa (1948) (Duplex-morph), *O. parmula* Corrêa (1950) (representative of the Fila morph), and *O. lactea* Corrêa (1954) (representative of the Lactea morph), all of them easily distinguishable morphologically. In this study, our main goals were: (1) to evaluate whether there is a shared demographic history for these four species that are found along 3,000 km of the Brazilian coastline; (2) to asses the degree of genetic structuring, with a special focus on the potential dispersal capability of these species. This is the first populational genetics study of marine meiofauna from South America.

Materials and methods

Sampling and DNA sequencing

All samples were extracted from sand samples collected from the intertidal zone following the method described by Corrêa (1949). *Ototyphlonemertes* specimens were found between March and October of 2006 in eight out of 25 visited localities encompassing 3,000 km of the coast (Fig. 1). Four *Ototyphlonemertes* species were sampled: *O. erneba* ($n = 59$; 5 localities), *O. lactea* ($n = 27$; 5 localities), *O. evelinae* ($n = 30$; 3 localities), and *O. parmula* ($n = 21$; 2 localities), a total of 137 specimens. Living specimens were identified using a dissecting microscope and a compound microscope, preserved in 95% ethanol, and kept at −20°C until the DNA extraction. Based on Envall and Norenburg (2001), the following morphological characters

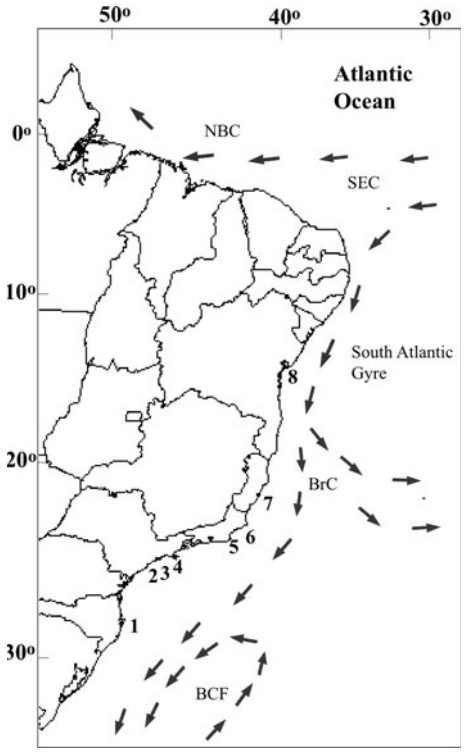

Fig. 1 Sampling localities and some major tropical South Atlantic Ocean currents. *1* Florianópolis (Fl); *2* São Sebastião (PV); *3* Ubatuba—south (BVS); *4* Ubatuba—north (VN); *5* Rio de Janeiro (Ver); *6* Macaé (Ma); *7* Vitória (Vi); *8* Salvador (FB). Currents abbreviations are: *NBC* North Brazil Current; *SEC* South Equatorial Current; *BrC* Brazil Current; *BCF* Brazil Current Front

for identifying species were used: (1) color of epidermis and brain region, (2) presence and position of the cephalic furrow, (3) presence and shape of cerebral organs, (4) proboscis papillae, (5) diaphragm size, (6) position and number of the accessory stylet pouches, (7) stylet structure, (8) shape of middle chamber, and (9) statolith type. We used a minimum of five traits to identify each individual, as some of the species share a common character state for one or more of these features.

Genomic DNA was extracted using the CTAB method described by Winnepenninckx et al. (1993), with modifications (see Thollesson 2000). For all specimens, an approximately 480-bp fragment of the mitochondrial cytochrome *c* oxidase subunit 3 (from now on COX3) gene was amplified using the primers COX3F (5′-TGCGWTG AGGWATAATTTTATTTATT-3′) and COX3R (5′-A CC AAGCAGCTGCTTCAAAACCAAA-3′) (Turbeville and Smith 2007). The polymerase chain reaction (PCR) cycling conditions were as follows: initial denaturation at 94°C for 1 min; 31 cycles of denaturation at 94°C for 30 s; annealing at 43–48°C for 1 min, extension at 72°C for 1 min; and final extension at 72°C for 3 min. PCR products were purified with ExoSAP-IT (USB). Sequencing reactions for both strands of amplified markers were run using BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems) and the PCR primers. Products were cleaned up using Sephadex columns, dried, and sequenced using an Applied Biosystems automated sequencer. The products were sequenced using CEQ dye terminator chemistry and a CEQ 8000 Genetic Analysis System (Beckman Coulter, Brea, California). The sequence contigs were assembled using Sequencher, version 4.5 (Gene Codes, Ann Arbor, MI). Blast searches (Altschul et al. 1997), as implemented in the NCBI website (http://www.ncbi.nlm.nih.gov), were checked for putative contamination. Sequences were deposited in GenBank under the accession number range HQ659778–HQ659847.

Data analysis

The alignment was performed by MUSCLE (Edgar 2004), and no length variation was observed. ModelTest 3.06 (Posada and Crandall 1998) module in HyPhy (Kosakovsky Pond et al. 2005) was implemented to choose the best fit model of molecular evolution for our data set under the Akaike Information Criterion (Akaike 1974). The test indicated the general time reversible model as best fitting our data with gamma shape parameter alpha to model rate heterogeneity (GTR + GAMMA). Phylogenetic trees were estimated for each species (from which duplicate sequences were removed) with 1,000 bootstrap replicates using the software RAxML v7.0.4 (Stamatakis 2006; Stamatakis et al. 2008). The search for the optimal Maximum Likelihood (ML) trees was performed on the Research Computing cluster odyssey facility from the Faculty of Arts and Sciences located at Harvard University. The ML tree search was conducted by performing 300 runs using the default algorithm of the program for random trees ($-d$ option) as a starting tree for each run. The final tree was determined by a comparison of likelihood scores under the GTR + GAMMA model among suboptimal trees obtained per run. Sequences from a species first identified as *Ototyphlonemertes fila*[1] in GenBank (accession numbers GU306064–GU306128) were added to the ML analysis to verify its placement on our gene tree, as another member of the phylomorph Fila. The palaeonemertean *Cephalothrix simula* was included as outgroup (sequence obtained at GenBank, accession number FJ594739). At the time of analysis, *Cephalothrix* was the only nemertean with a COX3 sequence available in the GenBank. Palaeonemerteans have a somewhat unclear position in the nemertean phylogeny, but there is little doubt about their basal position with respect to hoplonemerteans (Thollesson and Norenburg 2003; Andrade et al. subm).

[1] This *O. fila* designation was changed to *O. parmula* after this manuscript was accepted.

To describe genetic diversity of samples for each locality, number of polymorphic sites (S), haplotype diversity (h, Nei 1987), the nucleotide diversity (π, Tajima 1989), and the mean number of pairwise differences (k, Tajima 1983) were estimated using Arlequin version 3.1 (Excoffier et al. 2005) and DnaSP v5 (Librado and Rosas 2009). Haplotype diversity (h) and the nucleotide diversity (π) were tested for significant differences among species by a nonparametric Kruskal–Wallis test.

Networks of the mitochondrial COX3 haplotypes, which may be more appropriate than hierarchical trees for representing intraspecific evolution (Posada and Crandall 2001), were inferred using statistical parsimony (Templeton et al. 1992), as implemented in the program TCS v1.21 (Clement et al. 2000). The method links haplotypes with the smallest number of differences as defined by a 95% confidence criterion.

Mismatch distributions were used to evaluate the hypothesis of recent population growth with 10,000 permutations as implemented in Arlequin 3.1 (Rogers and Harpending 1992). This distribution is commonly unimodal in populations that have passed through a recent demographic expansion and is multimodal in stable populations. Mismatch distribution tests of spatial expansion were coupled with two other estimates of spatial or demographic expansion: Tajima's D (Tajima 1989), based on the number of segregating sites, and Fu's F (Fu 1996), based on the number of observed haplotypes and more sensitive than Tajima's D. Tajima's D statistic was developed to test for selective neutrality but can be used to test for population bottlenecks or rapid range expansions that also cause departures from equilibrium, where a non-significant D is consistent with a population at drift–mutation equilibrium (Tajima 1989). Fu's F tests were used to evaluate demographic population growth by detecting excesses of low-frequency haplotypes compared to the expected number in a static population. A significantly negative Fu's F is evidence of demographic growth. The significance was assessed with 10,000 permutations using Arlequin 3.1.

Arlequin (v3.1) was used to ascertain the degree of genetic differentiation among samples within each species through the analysis of molecular variance (AMOVA, Excoffier et al. 1992). A permutation test (10,000 randomizations) of genetic differentiation was calculated using the nearest-neighbor statistic (S_{nn}, Hudson 2000) implemented in DnaSP v5 (Librado and Rosas 2009). S_{nn} measures how often the most similar sequences in the sampling space (''nearest neighbors'') are from the same population, producing a powerful test of genetic differentiation for small data sets with rare haplotypes (Hudson 2000). Values of S_{nn} are expected to be near 1.0 for strong population differentiation, while an estimate of 0.5 would indicate that two groups are part of the same panmictic population. Pairwise genetic differentiation through S_{nn} was obtained, and its significance evaluated by 10,000 permutations with an alpha value adjusted by the Bonferroni correction for multiple tests (Rice 1989).

The relationship between genetic and geographical distances among localities of *O. erneba* was examined using reduced Major Axis (RMA) regression. Correlation of pairwise genetic distances ($S_{nn}/1 - S_{nn}$; Rousset 1997) and geographical distances among populations was assessed using the Mantel test (Mantel 1967) as implemented in the isolation-by-distance web service (IBDWS version 3.16; Jensen et al. 2005; http://ibdws.sdsu.edu/~ibdws/), with 30,000 permutation to test its significance.

The software Migrate-n version 3.0.3 (Beerli and Felsenstein 2001) was used to estimate long-term gene flow (averaged over the past n generations, where n = the number of generations populations has been at equilibrium) [$M = m/(m \times \mu)$, where m = migration rate and $(m \times \mu)$ = mutation rate] between population pairs and theta ($\Theta = N_e\ \mu$; where N_e = effective population size, and μ = mutation rate). This program estimates historical migration rates and effective population sizes using coalescence theory and Markov chain Monte Carlo techniques, assuming that populations are in migration-drift equilibrium. Parameter distributions were estimated using the maximum likelihood implementation of MIGRATE (Beerli 2008). Following a burn-in of 40,000, 2.5×10^6 genealogies were recorded at a sampling increment of 105 iterations. An adaptive heating scheme using 5 simultaneous Markov chains was implemented to increase the efficiency of searches. The program was run several times for each species using different random number seeds, and results were mostly stable, suggesting that Markov chains had converged on the stationary distribution. The same settings were used for all four species. The long-term gene flow estimate M was converted to the average number of immigrants per generation (xNm), using the formula $xNm = \Theta_{\ i} \times M_{i \to j}$. The nonparametric Kruskal–Wallis test was performed with BioEstat 5.0 (Ayres and Santos 2007) to evaluate significance of differences between the number of migrants per generation toward north and south, and between the number of migrants sent and received by locality. A pairwise Student–Newman–Keuls test followed by the Bonferroni correction (Rice 1989) was used to ascertain which pair of localities is responsible for a possible bias in the migration direction. Using ModelTest 3.06 (Posada and Crandall 1998), the transition/transversion ratio (ti/tv hereafter) and the base frequencies parameters were obtained for each species and incorporated in the analysis: *O. erneba*, $A = 0.45$, $C = 0.24$, $G = 0.14$, $T = 0.17$, ti/tv = 0.74; *O. evelinae*, $A = 0.45$, $C = 0.21$, $G = 0.13$, $T = 0.21$, ti/tv = 1.90; *O. lactea*, $A = 0.46$, $C = 0.22$, $G = 0.13$, $T = 0.19$, ti/tv = 1.24; *O. parmula*, $A = 0.47$, $C = 0.21$, $G = 0.14$, $T = 0.18$, ti/tv = 1.57.

Results

COX3 haplotypes relationships in *Ototyphlonemertes*

The 473-bp COX3 sequence revealed 169 polymorphic sites and 70 distinct haplotypes among 137 individuals. The results of the maximum likelihood analyses showed generally well-supported monophyletic groups in agreement with recognized species (ln $L = -4{,}815.04$), except for *O. parmula*. As expected (Envall and Norenburg 2001), *O. parmula* forms a clade with *O. fila.* That clade is sister to the rest of the group, with *O. evelinae* constituting the sister group of a clade composed of *O. lactea* and *O. erneba* (Fig. 2).

Ototyphlonemertes erneba samples yield 21 haplotypes with 56 polymorphic sites. The gene tree (ln $L = -1{,}459.95$, Fig. 3) shows no evidence of a well-supported monophyletic cluster defined by geographical structure, as relationships among collecting sites are largely unresolved, especially at the deep nodes. This same pattern is observed in *O. evelinae*; no well-supported clades related to geographic locality were found, and the deep nodes all show very low support (ln $L = -1{,}260.54$, Fig. 3). This species showed 35 polymorphic and 69 parsimony-informative sites in 22 exclusive haplotypes.

Ototyphlonemertes lactea presented 53 polymorphic sites. The phylogenetic tree (ln $L = -1{,}428.80$, Fig. 3) reveals two well-supported clades, one clustering samples from Vitória and the other encompassing all the other samples. The specimens collected at Vermelha constitute a well-supported clade. *Ototyphlonemertes parmula* showed also two separate well-supported clades, indicating strong geographic isolation (ln $L = -1{,}468.44$, Fig. 3). In fact, no haplotypes are shared between localities in this species. The 17 haplotypes obtained from a total of 21 specimens yield 28 polymorphic and 86 parsimony-informative sites (Fig. 4).

mtDNA diversity in *Ototyphlonemertes*

Table 1 shows the results of the diversity analyses for all species. Except for samples OlVer and OlVi, all other show moderate to high haplotypic diversity, ranging from 0.50 (OvVN) to 0.99 (OvFB), not statistically different among species (Kruskal–Wallis: $H_3 = 0.42$, $p = 0.93$). The nucleotide diversity is in general very low, with values between 0.001 (OvVN) and 0.066 (OlMa), and also not statistically different among species (Kruskal–Wallis: $H_3 = 5.3$, $p = 0.15$). The average pairwise nucleotide differences range from 0.50 (OvVN) to 31.3 (OlMa), indicating that the species have high range variation concerning intrapopulation genetic diversity estimates.

The statistical parsimony analysis connects all *O. erneba* haplotypes in a single network (Fig. 4a), with a few closed loops, which indicates some degree of homoplasy (Posada and Crandall 2001). Sixteen exclusive haplotypes were found, and the two most frequent haplotypes (15 and 18 specimens) are shared among all localities except Farol da Barra, which shares only one haplotype with Vitória. The two more frequent haplotypes account for 56% of the total number of specimens, whereas about 29% of the haplotypes are restricted to single localities.

A single network is also obtained for *O. evelinae* (Fig. 4a), where most of the haplotypes (about 77%) are restricted to single localities and only 5 out of 22 haplotypes are shared. The most frequent haplotype is shared

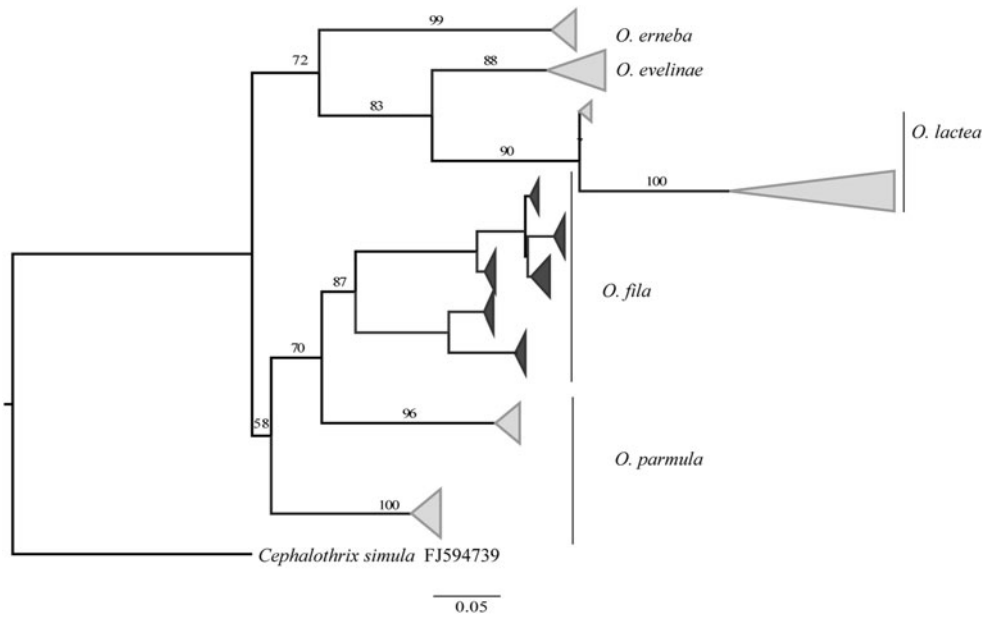

Fig. 2 Maximum likelihood gene tree of all *Ototyphlonemertes* species (ln $L = -4{,}815.04$). Three hundred searches and 1,000 bootstrap replicates were performed using the substitution model GTR + GAMMA. Numbers adjacent to nodes indicate bootstrap ≥50%. *Ototyphlonemertes fila* is represented by sequences deposited in Genbank (accession numbers GU306064–GU306128) by Tulchinsky et al. (in prep). *Cephalothrix simula* is the outgroup taxon

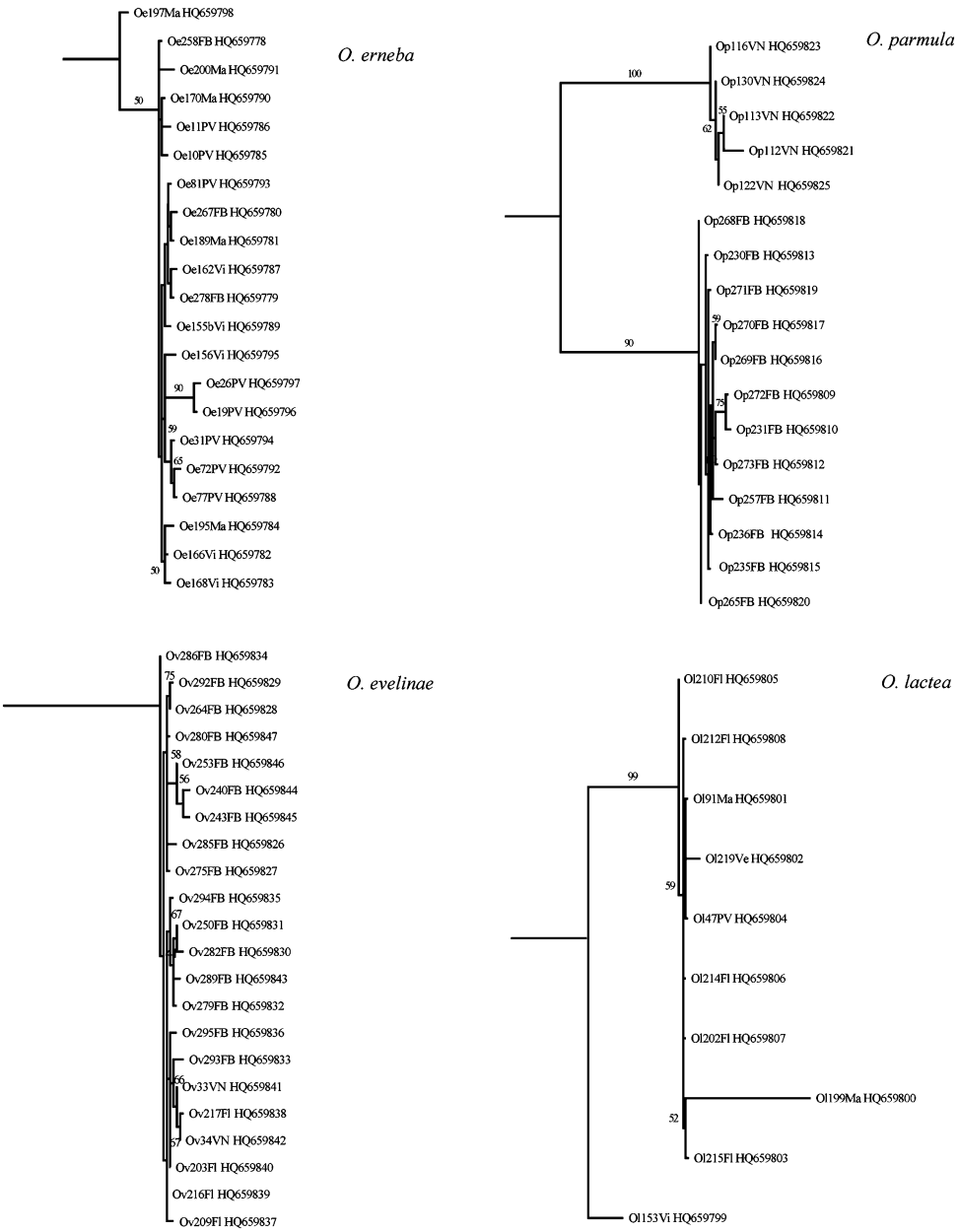

Fig. 3 Maximum likelihood gene trees of each *Ototyphlonemertes* species. Three hundred searches and 1,000 bootstrap replicates were performed using the substitution model GTR + GAMMA. Numbers adjacent to nodes indicate bootstrap ≥50%. All trees were rooted with *Cephalothrix simula*. Abbreviations on Table 1

between Vermelha do Norte and Farol da Barra, and the second most frequent is shared between Florianópolis and Farol da Barra, the two most distant localities (about 3,000 km).

TCS analysis of *O. lactea* resulted in three distinct networks (Fig. 4a), encompassing 10 haplotypes, none of them shared among localities. The networks cannot be connected within the limits of parsimony (9 mutational steps at 95% confidence). Clade A, which includes most of the haplotypes (not shared by different locations), represents 19 individuals from the localities OlMa, OlVer, OlFl, and OlPV. Macaé (OlMa) is represented by only two haplotypes (clade B), and when the TCS significance limit is relaxed, the connection between clades A and clade B requires 43 mutational steps. These two haplotypes differ in 47 bp, about 10% of the total COX3 fragment length. The Vitória sample (OlVi, clade C) is represented by just one haplotype and also requires a relaxed significance limit to connect, by 36 mutational steps, to clade A.

The TCS parsimony analysis of *O. parmula* also resulted in two non-connected networks by the limits of parsimony (12 mutational steps at 95% confidence). The two networks correspond to the sampling localities Salvador (OpFB, clade A) and Ubatuba-north (OpVN, clade B) (Fig. 4a). Only two haplotypes are shared among individuals, representing 28% of the total number of specimens.

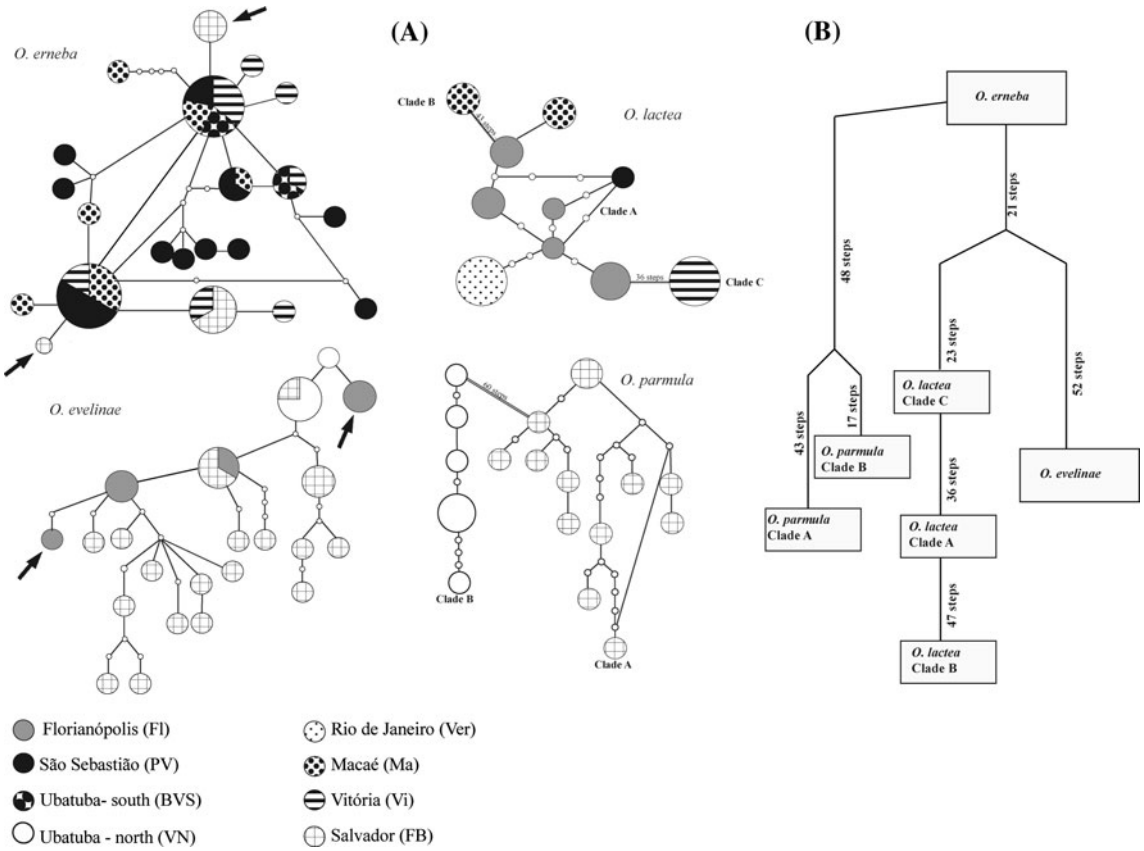

Fig. 4 **a** Most parsimonious haplotype networks (estimated cladogram) for the COX3 haplotypes of species of *Ototyphlonemertes.* Connection steps have a 95% probability of being linked without homoplasy. *Solid lines* connect haplotypes with a single step (missing intermediates are indicated by an *open circle*). The size of the circle is proportional to the number of represented haplotypes. See Table 1 for the localities abbreviation. *Arrows* indicate points of support for gene flow in *O. evelinae* and *O. erneba*. **b** Simplified network among species. The *rectangles* represent the clades obtained using the 95% confidence criterion. The *clade letters* correspond to **a**

The TCS analysis was also performed with all species with relaxed significance limit as the networks could not be connected within the limits of parsimony (11 mutational steps at 95% confidence). The network (Fig. 4b) presents *O. erneba* connected with all other species, and no connection whatsoever was observed among *O. lactea*, *O. parmula*, and *O. evelinae*. A minimum of seventy-three mutational steps was observed between *O. evelinae* and *O. erneba*. Forty-four mutational steps were observed between *O. erneba* and *O. lactea* clade C, which was connected to *O.lactea* clade A by 36 mutational steps; clade A was then connected to clade B by 47 mutational steps. The branch between *O. erneba* and *O. parmula* clade A requires 91 mutational steps, while between *O. erneba* and *O. parmula* clade B, 65 mutational steps. Figure 4b presents a simplified network among clades represented by rectangles. *Ototyphlonemertes evelinae* and *O. erneba* each have single haplotype networks; therefore, they are represented by just one rectangle.

AMOVA analyses resulted in different outcomes for the Brazilian *Ototyphlonemertes* species (Table 2). *Ototyphlonemertes erneba* displays concordant significance but low F_{ST} and S_{nn} values, indicating panmixia among sampled localities. The only significant pairwise S_{nn} estimate was between samples OeMa and OeFB (about 1,500 km apart). *Ototyphlonemertes erneba* populations do not show correlation between genetic and geographical distance (Mantel test $r = 0.65$; $p = 0.08$). *Ototyphlonemertes evelinae* also displayed more variation within localities than among them (Table 2), with significant structuring (F_{ST} and S_{nn}, respectively, 0.132 and 0.72) and, except for OvFl and OvFB, all pairwise S_{nn} values were high and significant (Table 3). Both *O. lactea* and *O. parmula* showed high and significant structuring (Table 2). *Ototyphlonemertes lactea* displayed more variation among populations (58%) than within populations (42%), indicating overall low intrapopulational variability and low probability of gene flow. Pairwise S_{nn} estimates of *O. lactea* were all very high and significant (Table 3).

None of the Tajima's D values were statistically significant for any species, except when *O. erneba* was analyzed as a whole clade. However, the populations OeMa,

Table 1 Cytochrome *c* oxidase 3 variation and diversity indexes for *Ototyphlonemertes* samples

Species	Sample (*n*)	*H*	*S*	*h* (SD)	π (SD)	*k* (SD)
O. erneba	OePV (22)	10	11	0.80 (0.72)	0.004 (0.0010)	3.08 (1.6)
	OeBVS (5)	2	6	0.60 (0.17)	0.007 (0.0020)	3.60 (2.1)
	OeMa (13)	6	19	0.96 (0.49)	0.010 (0.0020)	4.39 (2.3)
	OeVi (14)	6	12	0.94 (0.04)	0.007 (0.0010)	2.83 (1.5)
	OeFB (5)	3	8	0.80 (0.16)	0.010 (0.0020)	4.40 (2.6)
O. lactea	OlFl (9)	5	6	0.86 (0.08)	0.040 (0.0006)	1.94 (1.2)
	OlVer (6)	1	0	–	–	–
	OlMa (4)	2	47	0.66 (0.20)	0.066 (0.0200)	31.33 (17.4)
	OlVi (7)	1	0	–	–	–
O. evelinae	OvFl (6)	3	7	0.86 (0.12)	0.007 (0.0010)	3.4 (2.0)
	OvVN (4)	2	1	0.50 (0.26)	0.001 (0.0005)	0.5 (0.5)
	OvFB (20)	18	27	0.99 (0.19)	0.010 (0.0010)	6.12 (3.0)
O. parmula	OpVN (8)	5	11	0.78 (0.15)	0.007 (0.0020)	3.39 (1.9)
	OpFB (13)	12	17	0.98 (0.03)	0.010 (0.0010)	4.43 (2.3)

n Sample size, *H* number of haplotypes, *S* number of polymorphic sites; *h* haplotype diversity, π nucleotide diversity; *k* average number of pairwise differences, *SD* standard deviation

Table 2 Analysis of molecular variance (AMOVA) and its significance based on 16,000 replicates

Species	Hierarchical level (*df*)	Variance	% Total	F_{ST}	S_{nn}
O. erneba	Among populations (4)	0.029	5.94	0.059**	0.33**
	Within populations (54)	0.475	94.06		
O. lactea	Among populations (3)	0.295	58.24	0.582***	1.00***
	Within populations (22)	0.212	41.76		
O. evelinae	Among populations (2)	0.069	13.22	0.132***	0.72**
	Within populations (27)	0.456	86.78		
O. parmula				0.106**	1.00***

df Degrees of freedom

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

OePV, OeVi, OlFl OvFB, and OpFB displayed negative and significant Fu's *F* test (Table 4), rejecting null hypothesis of selective neutrality and indicating selection, bottleneck or population expansion. Also, analyzing *O. evelinae* and *O. erneba* each as a whole clade yielded negative and significant *F* tests.

Mismatch distribution was unimodal for most of the localities (Table 4), consistent with of the model of sudden expansion from a small number of specimens. The sum of square deviations (SSD) test suggests that the observed distribution curves do not significantly differ from the simulated distribution curves under a model of sudden expansion after a bottleneck for these localities. Only samples from OeBVS, OlFl, OlMa, and *O. lactea* clade A showed significant deviation for the null hypothesis (Table 4). The rejection of the sudden-population-expansion hypothesis in OlMa is due to the large difference between the two haplotype lineages (47 variable sites), resulting in a very old coalescent event.

Results of the MIGRATE analyses indicated that *Ototyphlonemertes erneba* populations share migrants mostly symmetrically, and no significant direction pattern is detected toward north or south (Kruskall Wallis $H_1 = 0.89$, $p = 0.34$, Table 5). There was statistical support for differences in the average of migrants per generation sent by each locality (Kruskall Wallis $H_4 = 16.8$, $p = 0.002$). The Student–Newman–Keuls test shows significant pairwise differences between OeMa and the localities BVS and FB ($p < 0.001$). There was no significant difference between the average of migrants per generation received in each locality for *O. erneba* (Kruskall Wallis $H_4 = 0.4$, $p = 0.98$). *Ototyphlonemertes evelinae* shows asymmetric migration southward, especially from OvFB to OvVN ($\sim$23 migrants per generation), about 2,000 km distant

Table 3 Intraspecific pairwise S_{nn} estimates per species with significance levels based on 10,000 permutations (after Bonferroni correction, Rice 1989)

Samples	OeBVS	OeMa	OeVi	FB
OePV	0.740	0.500	0.572	0.740
OeBVS	–	0.780	0.709	0.800
OeMa	–	–	0.523	0.861**
OeVi	–	–	–	0.807
	OlVer	OlMa	OlVi	
OlFl	1.000***	1.000**	1.000***	
OlVer	–	1.000**	1.000***	
OlMa	–	–	1.000**	
	OvVN	OvFB		
OvFl	0.960*	0.770*		
OvVN	–	0.900**		
	OpFB			
OpVN	0.916***			

– Not applicable. Abbreviations on Table 1

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

from each other. *Ototyphlonemertes lactea* showed virtually no gene flow, with the mean number of migrants under one per generation (Table 5). *Ototyphlonemertes parmula* also barely shared migrants (21×10^{-2} migrants per generation from OpFB to OpVN and none the other way around).

Discussion

Ototyphlonemertes genetic variability and demographic history

Previous genetic surveys have shown the existence of cryptic lineages in nemerteans (Strand and Sundberg 2005a; Mateos and Giribet 2008; Sundberg et al. 2009; Chen et al. 2010; Mahon et al. 2010). In the present study, the mitochondrial gene COX3 produced a mosaic of outcomes with respect to differentiation within and among *Ototyphlonemertes* species, including potential cryptic speciation in *O. lactea* and *O. parmula* (Figs. 3, 4a).

Ototyphlonemertes lactea data yielded three highly divergent clades (Fig. 4), one composed exclusively of specimens collected at Vitória (OlVi), the northernmost location sampled for the species. This location, represented by a single haplotype, could be isolated due to restricted gene flow, which also would partially explain its lack of variability (Table 1). This could be explained by the prevailing direction of the Brazilian oceanic currents (Fig. 1), which would bring larvae or adults southward. Two of four *O. lactea* individuals from Macaé are connected to the main haplotype network only when the parsimony confidence limit is relaxed (Fig. 4a). All four were collected on

Table 4 Neutrality tests and demographic history estimates on cytochrome *c* oxidase 3 for *Ototyphlonemertes* samples

Species	Samples	*D*	*F*	Mismatch	τ	θ_0	θ_1
O.erneba	PV	−0.96	**−26.29*****	3.1 ± 2.6	1.52	1.75	11.3
	BVS	1.72	3.96	**3.6 ± 3.1**	**7.12***	**0**	**12.4**
	Ma	−1.20	**−7.29*****	4.4 ± 3.0	2.97	0	99,999
	Vi	−0.99	**−11.57*****	2.8 ± 1.7	2.88	0	46.1
	FB	1.03	2.05	4.4 ± 2.7	6.93	0	27.7
	All samples	**−1.83***	**−26.4*****	1.3 ± 2.6	1.3	1.17	99,999
O. lactea	Fl	−0.52	**−2.25***	**1.9 ± 0.89**	**2.52***	**0**	**99,999**
	Ma	2.31	8.72	**31.3 ± 24.2**	**47***	**0**	**2.7**
	Clade A	−0.12	−2.2	**2.27 ± 1.2*****	**2.9**	**0**	**99,999**
O. evelinae	Fl	0.63	0.46	3.4 ± 2.3	5.46	0	7.8
	VN	−0.61	0.17	0.5 ± 0.5	0.76	0	99,999
	FB	−1.16	**−10.98*****	6.16 ± 3.5	5.44	0	119.8
	All samples	1.35	**−16.4*****	5.4 ± 3.3	3.8	1.5	49.2
O. parmula	VN	−1.00	−0.02	3.39 ± 3.0	2.08	2.2	5.68
	FB	−0.80	**−7.24*****	4.43 ± 2.5	2.2	2.71	99,999

D Tajima's *D*, *F* Fu's *F* test, *Mismatch* mean and standard deviation of mismatch distribution, τ units of divergence time; θ_0 function of population size before expansion, θ_1 function of population size after expansion. Abbreviations on Table 1

The values in bold are statistically significant

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 5 Average of migrants per generation of *Ototyphlonemertes* species obtained using MIGRATE

	Receiving location Sender location	OePV	OeBVS	OeMa	OeVi	OeFB
O. erneba	OePV	–	1.92 (0.8)	2.11 (0.9)	2.45 (0.9)	2.3 (1.0)
	OeBVS	1.37 (0.4)	–	1.48 (0.7)	1.77 (1.4)	1.33 (0.3)
	OeMa	4.13 (3.2)	2.78 (0.7)	–	3.41 (1.3)	4.27 (0.4)
	OeVi	1.95 (0.9)	1.89 (0.6)	2.11 (1.3)	–	1.94 (1.4)
	OeFB	1.81 (0.3)	1.40 (0.1)	1.54 (0.4)	1.3 (0.1)	–
O. lactea		OlFl	OlVer	OlMa	OlVi	
	OlFl	–	0 (0.0)	0 (0.0)	0 (0.0)	
	OlVer	7×10^{-2} (0.1)	–	16×10^{-2} (0.3)	0 (0.0)	
	OlMa	44×10^{-2} (0.4)	0 (0.0)	–	0 (0.0)	
	OlVi	8×10^{-2} (0.1)	0.2×10^{-2} (0.0)	0 (0.0)	–	
O. evelinae		OvFl	OvVN	OvFB		
	OvFl	–	0 (0.0)	0 (0.0)		
	OvVN	2.13 (1.6)	–	0 (0.0)		
	OvFB	0 (0.0)	22.9 (11.8)	–		
O. parmula		OpVN	OpFB			
	OpVN	–	0 (0.0)			
	OpFB	21×10^{-2} (0.0)	–			

The results are mean values of 13 runs for *O. erneba*, 7 runs for *O. lactea*, 3 runs for *O. parmula*, and 8 runs for *O. evelinae*. The standard deviation is in parentheses. Abbreviations on Table 1

the same day and at the same spot and identified based on the same traits. Though the individuals are morphologically similar to all other *O. lactea* collected, they seem to represent a distinct and rapidly evolved lineage, as evidenced by the high number of accumulated mutations. This is supported by the mismatch distribution analysis (Table 4) showing very high average pairwise differences within this cluster (=31.3). The three clades can be characterized as distantly related, with the number of mutational steps sometimes higher than the difference among species (Fig. 4b). For example, *O. erneba* and the *O. lactea* clade C are separated by 44 mutational steps, while *O. lactea* clade B and C are separated about 83 mutational steps. These results reinforce the hypothesis that the samples collected as *O. lactea* might constitute three cryptic species. Morphologically similar but genetically distinct lineages that are not isolated geographically are common in nemerteans, as pointed out by Chen et al. (2010 and references therein), and are attributed to cryptic speciation.

The specimens of *O. lactea* from Florianópolis showed higher diversity than the samples from other locations (Table 1). Results of the mismatch distribution (SSD $p = 0.02$) and the Fu's F test ($p = 0.03$) were contradictory: mismatch distribution did not support the population expansion model, while Fu's F test provided evidence for population growth. Star-shaped gene genealogies and significant negative values in neutrality tests result after a range expansion, but only if the demes exchange a high number of migrants (Ray et al. 2003). Ray et al. (2003) suggested that the hypothesis of selective neutrality would be rejected more often for relatively recent expansions ($\tau < 3$) than for older expansions ($\tau > 5$). Thus, the Florianópolis population (OlFl) might have experienced some spatial expansion, with, however, a very low number of migrants (Ray et al. 2003; Magoulas et al. 2006). The other hypothesis to explain this contradictory result is positive selection, which would explain the negative value (Fu 1997). However, based on our fieldwork observations, *O. lactea* populations seem to be very small, and consequently more prone to stochastic fluctuations than to natural selection. When the whole *O. lactea* clade A is analyzed, the mismatch distribution test has a statistically significant result, but not the neutrality test, suggesting that the population size in the whole clade is constant. OlVi and OlVer locations were composed of just one haplotype, which might mean that these populations result from recent colonization or experienced a recent severe bottleneck event. If the latter, it would explain the significant SSD test for clade A, suggesting reduction of its effective population size.

In some organisms, occasional extinction and recolonization can enhance the rate of differentiation between demes (Wright 1977; Casu and Curini-Galletti 2006; Derycke et al. 2007), which could explain the high genetic divergence among sampled *O. lactea* lineages. Ibrahim et al. (1996) showed that subdivided populations could occupy a continuous habitat as a result of stochasticity in dispersal and settlement. In this case, even high dispersal rates lead to the establishment of several discrete groups. Some studies have addressed the meiofauna ecological dynamics in Brazil and concluded that mostly abiotic factors like rainfall, grain size, salinity, dissolved oxygen, among others, influence greatly the spatial and temporal distribution and recruitment of interstitial organisms (Somerfield et al. 2003; Souza-Santos et al. 2003; Fonsêca-Genevois et al. 2006; Albuquerque et al. 2007). In natural populations, temporal and spatial effects cannot be disentangled and, sometimes, temporal variation enhances effects of spatial variation on distribution of genetic variability, even at a small scale (Bryant 1976; De Wolf et al. 1998). It is uncertain to what degree these same abiotic factors are influencing the distribution and settlement of *O. lactea*, but considering how these dynamics can affect co-inhabiting meiofauna, they are likely to play a role in establishment of *O. lactea* populations.

As in *O. lactea*, it is very clear that the two *O. parmula* lineages are geographically defined (Fig. 4a, b). *Ototyphlonemertes parmula* samples were only found in Salvador and Ubatuba, about 2,000 km apart. On the other hand, population OpFB is sister group of *Ototyphlonemertes fila*, which is not surprising, in light of evidence that *O. fila* also might be composed of several cryptic lineages (Tulchinsky et al. in prep). With only two locations yielding specimens and only one variable marker, it is difficult to ascertain whether we are dealing with one species or two cryptic species. The number of missing haplotypes or mutational steps is similar or higher than the difference among some of the *Ototyphlonemertes* analyzed (Fig. 4b), strongly suggesting that we are dealing with two cryptic species. By the chosen diagnostic characters, samples from both locations agree with the description by Corrêa (1950). The first and only previously documented occurrence of *O. parmula* was at its type locale in Ilhabela (São Sebastião), less than 100 km far from Ubatuba, so one would expect that the clade found in Ubatuba corresponds to the type described by Corrêa (1950). Unlike *O. lactea*, *O. parmula* showed a high diversity of haplotypes within locations (Table 1), especially in OpFB, and both populations seem to have concordant results between the Fu's test of neutrality and the mismatch distribution, indicating sudden population expansion after bottleneck. Both populations have a similar value of divergence time (τ, Table 4), suggesting a common and relatively recent expansion time.

The low level of structuring seen for *O. erneba* (Tables 2, 3) indicates little genetic differentiation among localities, even among those several hundreds of kilometers apart (e.g., OePV and Vi, non-significant $S_{nn} = 0.57$, $\sim$1,000 km apart). Overall, most of the variation ($\sim$94%) was found within localities, and such low values of F_{ST} and S_{nn} indicate panmixia along the Brazilian coast. This is confirmed by the haplotype network (Fig. 4a), which shows that the most common haplotypes are shared among the different sampled locations, and there is no evident geographic isolation. The species *Ototyphlonemertes erneba* presented a picture of sudden expansion, with a star-shaped network, confirmed for both neutrality tests, except for OeBVS.

In general, *Ototyphlonemertes evelinae* presented a pattern similar to *O. erneba*: significant but moderate structuring, high genetic variation within populations ($\sim$87%) and shared haplotypes among locations. Once again, there was no clear geographic pattern; the most distant locations (OvFl and OvFB, $\sim$2,700 km apart) shared haplotypes, and no common haplotypes were observed between OeFl and OvVN ($\sim$800 km apart). OvFB presented a significant signal of spatial expansion, confirmed for both Fu's *F* and the mismatch distribution test (Table 4). Indeed, OpFB and OpFl showed a higher value of divergence time ($\tau > 5$, compared to 0.76 for OpVN), which suggests that these populations are older and explains their high number of unique haplotypes. Recurrent colonization could maintain rare alleles in very large populations, when followed by a subsequent and short bottleneck (Zbawicka et al. 2003). In marine organisms, repeated extinction/recolonization events are assumed to be the main processes involved in producing similar amounts of spatial and temporal genetic differentiation in some species, as seen in the hydrothermal vent populations of the polychaete *Alvinella pompejana* (Jollivet et al. 1998). One can reasonably expect this to be the case for beaches subjected to regular or occasional high-energy wave action. So, while populations are disjunctively distributed, they simultaneously seem to share organization of intrapopulational genetic diversity. In other words, while there is genetic differentiation among populations, the way the genetic diversity is distributed and maintained is homogeneous among populations that occur in the same environment, due to recurrent events of extinction/recolonization, even with low gene flow within species. There is no statistically significant difference in the intrapopulation diversity indexes, i.e., of haplotype and nucleotide diversity among species. The only exception is observed in *O. lactea*, where the locations OlVi and OlVN show no variability at all, probably caused by recent colonization associated with apparent lack of gene flow.

Larval dispersal in *Ototyphlonemertes*?

In the marine environment, boundaries within species' distributions may either be generated by (1) hydrodynamic factors limiting recruitment or availability of planktonic larvae (e.g., Gaylord and Gaines 2000; Hohenlohe 2004) or (2) gradients in ecological factors that can impose limit ranges (Lee and O'Foighil 2005; Rocha et al. 2005). Few studies of nemerteans have addressed gene flow, but there is evidence of dispersal irrespective of developmental type. For example, the broadcast-spawning *Parborlasia corrugata* presented a mitochondrial haplotype structure pattern coherent with dispersal (Thornhill et al. 2008), while *Lineus ruber* and *L. viridis*, direct developers, showed only moderate structuring and very high genetic identity in populations sampled across the Atlantic Ocean (Rogers et al. 1998).

Ototyphlonemertes, like most hoplonemerteans, are dioecious and at least a few are known to have a lecithotrophic planuliform larva with a pair of eyes and statocysts (Chernyshev 2000; Norenburg and Stricker 2002). All adult *Ototyphlonemertes* lack eyes; their presence in larvae can reasonably be inferred to indicate that a pelagic phase is normal, not just the result of being washed into the plankton. Apparently, hoplonemerteans only start feeding once the proboscis is functional (Iwata 1960 in Norenburg and Stricker 2002; JLN pers obs), so a planktonic phase is constrained by yolk reserves, and it is unlikely that the larvae could have a long-term dispersal. In fact, *Ototyphlonemertes* larvae are known only from near-shore plankton (Jägersten 1972, fig 20; Chernyshev 2000). Based on the expected low capacity for long-term dispersal in *Ototyphlonemertes* species, not finding evidence of gene flow, as observed in *O. lactea* and *O. parmula*, is not surprising. However, slight differences occurred between the two species: in *O. parmula* it is plausible that there are two cryptic lineages, genetically very divergent, with an incipient signal of gene flow from OpFB to OpVN, an average of less than one migrant per generation. *Ototyphlonemertes lactea* also showed low gene flow, which agrees with the lack of observed shared haplotypes. These results are expected, and the observed weak migration sign observed might be due to passive dispersal, such as rafting (Jokiel 1990), with adult nemerteans transported along with or separated from sediment in the water column. Dispersal by means of rafting is likely to be sporadic, and result in low levels of gene flow among populations, with high potential for founder speciation (Paulay and Meyer 2002).

Surprisingly, *O. evelinae* and *O. erneba* revealed a contrasting scenario, with evidence of long-term gene flow (Table 5) among locations thousands of kilometers apart. The high number of migrants per generation can hardly be explained by occasional adult migration, which might suggest that larval migration plays a role in these species. Asymmetric migration toward the south was observed in *O. evelinae*, from OvFB to OvVN and from OvVN to OvFl, which is consistent with the major currents along the Brazilian coast (Cirano et al. 2006; Fig. 1). There is also evidence of dispersal in the haplotype network in *O. evelinae* (Fig. 4): one OvFl haplotype is more similar to OvFB and OvVN haplotypes than other OvFl haplotypes. The same can be observed for some OvFB haplotypes, which are more similar to OvFl haplotypes. *Ototyphlonemertes erneba* presents the same pattern for some OeFB haplotypes and one OePV haplotype (Fig. 4). In *O. erneba*, there is no visible directional migration pattern, but migration seems to be frequent and, in general, symmetrical, despite the evidence that more migrants per generation are sent by OeMa than by the other locations (Table 5). Some authors proposed that frequent episodes of extinction and recolonization can lead to populations with low difference in gene frequencies (Slatkin 1987; Barton and Whitlock 1997). This assumption makes sense if accompanied by constant gene flow in *Ototyphlonemertes erneba* and the populations are panmictic (Tables 3, 5).

How does one explain the disparity in structuring and demographic history among the Brazilian *Ototyphlonemertes* species? At each location, the species were collected in the same small area, so it is likely that they were subjected to the same environmental conditions. Species-specific differences in larval duration as well as specific requirements concerning habitat characteristics could explain the differences, as observed in other marine species (Reid et al. 2006; Bird et al. 2007; Crandall et al. 2008). Congeneric variation in larval development is not uncommon in nemerteans, as observed in *Lineus* (Rogers et al. 1998); however, it is not commonly observed in hoplonemerteans. Reproductive biology in *Ototyphlonemertes* can vary among species, with some species filling the body with many ($\sim$40–100) synchronous oocytes while other, sympatric, forms may have relatively few ($\sim$4–10) oocytes maturing simultaneously (JLN pers obs). This could play an important role in the population effective size, and thereby account for the differences observed at the inter-population level among species. Also, weather events, like storms, can differentially affect species that maintain intrinsically different population sizes, in that populations could differ in fitness and therefore success, abundance, and sensitivity to local extinction. This effect would play a stochastic role on the distribution of genotypes and could partially explain the lack of concordance in distribution patterns. For instance, Corrêa (1950) describes *O. parmula* as having a caudal adhesive plate, a feature unique to some *Ototyphlonemertes* species. Its presence is consistent with the occurrence of adhesive specializations among interstitial fauna and believed to facilitate their ability to remain in

the sediment during disturbance (Swedmark 1964). Nevertheless, *Ototyphlonemertes* species with and without caudal adhesive plates often co-occur in the same samples. We noted presence of a caudal adhesive plate for *O. parmula*, but it can be exceedingly difficult to detect (JLN pers obs), and reported or inferred absence always must be accompanied by some doubt. Corrêa (1953) notes that the other three species, while lacking a caudal adhesive plate, also display strong haptic reactions to hydrodynamic disturbance. We cannot rule out differences in haptic or other behavioral responses, but these do not seem likely to explain the species differences seen in this study. Further studies on these species are needed to determine more fully the life-history parameters of all the species, and particularly whether *O. erneba* and *O. evelinae* larvae are more capable of long-term dispersal compared to the other Brazilian *Ototyphlonemertes*.

Concluding remarks

Our gene tree shows that the Lactea and Pallida morph are sister groups in a clade with a Duplex-morph, and Fila morph would be the basal group. This does support the hypothesis presented by Envall and Norenburg (2001), but implies coordinate polyphyletic origin of a spirally sculpted stylet and polygranular statocyst. However, the present study is limited to just one gene, which limits our conclusions about species delimitation. A study on the phylogenetic relationship and species boundaries within the *Ototyphlonemertes* using several molecular and morphological markers is underway and will be able to shed some light on the biological significance of the phylomorphs (Andrade and Norenburg, unpubl data).

Except for *O. parmula*, *Ototyphlonemertes* species showed monophyly in the explored mtDNA lineages (Fig. 2). All phylomorphs (Envall and Norenburg 2001) seem to be monophyletic, taking into account that the cryptic lineages found are also part of the phylomorph previously determined. Finding cryptic lineages is not surprising in light of several studies on nemerteans showing lack of concordance between morphological and molecular diversity (e.g., Envall and Sundberg 1998; Strand and Sundberg 2005a, b; Sundberg et al. 2009; Chen et al. 2010 and references therein; Sundberg and Strand 2010). Clearly, the use of morphological traits alone is impractical for characterizing *Ototyphlonemertes* species. In fact, Sundberg and Strand (2010) questioned the use of internal anatomy as a tool in nemertean taxonomy and phylogeny, due to the difficulty and inconsistency in obtaining reliable information from these internal characters. However, among nemerteans, *Ototyphlonemertes* is one of the easiest genera to diagnose, and its species exhibit the largest suite of discontinuously variable and otherwise differentiable morphological characters, most of which can be discerned from living specimens. Although the smallest monophyletic units are difficult to delimit based on morphological characters alone (Envall and Norenburg 2001), the accessibility of those characters and the large number of morphotypes (ibid) make the genus very attractive for a variety of potential evolutionary studies. It is evident from this study that not all morphotypes are abundant, but there are many places in the world where one or several are both abundant and spread over wide geographic range (ibid; JLN unpublished data).

There is a dearth of fine-scale ecological studies that focus on unraveling the extent, the organization and the boundaries of genetic diversity in *Ototyphlonemertes* and for meiofauna in general. This study shows that despite similar ecologies and generally sympatric distributions along the Brazilian coast, the congeneric taxa *Ototyphlonemertes erneba*, *O. evelinae*, *O. lactea*, and *O. parmula* have unique genetic diversity patterns for the COX3 fragment, and their phylogeographic profiles differ sharply. Although the patterns shown here are based on a mitochondrial gene, the present study brings new insights as the first genetic population study of Brazilian meiofauna, and the first comparative population-level study of *Ototyphlonemertes* across such a large latitudinal range.

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