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Are meiofaunal species cosmopolitan? Morphological and molecular analysis of *Xenotrichula intermedia* (Gastrotricha: Chaetonotida)

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Abstract Many meiofaunal species are reported to be cosmopolitan, but due to uncertainties of identification, the affiliation of specimens from geographically distant areas to the same species-taxon is problematic. In this study, we examined morphological and molecular variation in samples of *Xenotrichula intermedia* Remane (Gastrotricha: Chaetonotida) from the Mediterranean Sea, the northwestern Atlantic and the northern Gulf of Mexico. Univariate analysis of 16 morphological traits was unable to detect differences among populations, except for the length of the pharynx, which was significantly shorter in the Gulf of Mexico specimens. Canonical discriminant analysis separated the Gulf of Mexico specimens from the other two populations, with pharynx length contributing about half of the total discrimination. Molecular analysis based on restriction-fragment length polymorphisms (RFLPs) in a 710-base pair polymerase chain-reaction (PCR) product representing roughly half of the mitochondrial cytochrome oxidase I (COI) gene detected four haplotypes: one each from the Mediterranean and the Gulf of Mexico populations and two coexisting within the Atlantic population. The estimated nucleotide-sequence divergence calculated for each pairwise combination of haplotypes (based on the proportion of shared fragments) ranged from 5.3 to 11.5%. The high genetic divergence and the inability to clearly separate populations based on morphology suggest that individuals characterized by different haplotypes are genetically isolated sibling species.

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Introduction

Meiofaunal species are defined as cosmopolitan if they are reported from two or more oceans, including connected seas (Sterrer 1973). Within the meiobenthos, cosmopolitan species have been reported from a wide array of major taxa displaying a broad range of life styles (Nematoda: Gerlach 1962; Ostracoda: Hulings 1971; Polychaeta: Westheide 1971; Tardigrada: Renaud-Mornant and Pollock 1971; Harpacticoida: Wells 1986; Gastrotricha: Hummon et al. 1994; Gnathostomulida: Sterrer 1973). Although frequently reported, the high proportion of cosmopolitan species has been questioned, because (1) distributional records usually come from different sources at different times, and (2) the number of specimens examined is generally too low to allow meaningful comparisons within and among populations (see also Sterrer 1973). Indeed, because of shared life-history traits such as a short life cycle, a relatively small number of offspring, the general absence of a pelagic larval stage, and a relatively limited swimming ability of adults, meiofaunal species (particularly interstitial forms) would be expected to have restricted geographical ranges.

A central point of the debate over the presumed cosmopolitan distribution of meiofaunal taxa concerns species identification. In particular, critics have questioned the reliability of species identifications from geographically distant areas, especially when made by different investigators using different methods (often low-resolution microscopy) and probable personal "instincts" to affiliate specimens with a given taxon. In fact, careful morphological analysis has shown that some species with a presumed wide geographic range are actually composite assemblages of different species

(R. Huys, cited in Giere 1993; Evans 1994). On the other hand, recent surveys using highly reproducible techniques (e.g. high-resolution video microscopy) have confirmed that cosmopolitanism appears to be a widespread phenomenon among certain meiofaunal groups (Hummon 1994; Todaro et al. 1995). Because of these contrasting results and the awareness of the possible existence of cryptic species, different approaches to species identification are needed to either confirm or disprove the morphological species concept. Physical characteristics of the interstitial habitat have prevented direct observations of behavioral/ecological differences among geographically distant populations. Moreover, small body size and the scarcity of specimens from single sites have, in most cases, prevented a more accurate species identification based on a genetic approach. The polymerase chain reaction (PCR: Saiki et al. 1988) allows one to amplify large quantities of specific DNA sequences from very small initial amounts of template (i.e., Harris et al. 1990). The PCR products can subsequently be analyzed for nucleotide sequence differences and/or restriction-fragment length polymorphisms (i.e., Gárate et al. 1991; Karl and Avise 1993; Castagnone-Sereno et al. 1994; Geller et al. 1994; Litvaitis et al. 1994).

Here we focus on Gastrotricha (using both morphological and molecular genetic analysis) to examine the paradoxical existence of cosmopolitan meiofaunal species. Species of this phylum are ubiquitous in unpolluted sandy habitats, where gastrotrichs often rank second or third in abundance after nematodes and harpacticoid copepods (Coull 1988). A fairly high number of gastrotrichs are reported as multi-regional cosmopolitans (i.e., Ruppert 1977; Hummon et al. 1994; Todaro et al. 1995). We investigated *Xenotrichula intermedia* Remane, a small interstitial chaetonotid characteristic of (but not limited to) the intertidal zone of high-energy beaches, that is frequently reported in faunal surveys of coastal areas in northern Europe, across the Mediterranean Sea, the Atlantic and Gulf coasts of the USA, and also from India and Somalia (i.e., Ganapati and Rao 1967; Balsamo et al. 1992; Jouk et al. 1992; Todaro et al. 1995). We compared specimens from the Mediterranean Sea, the northwestern Atlantic, and the northern Gulf of Mexico. At the morphological level we measured 16 traits, whereas at the molecular level we focused on a portion of the cytochrome oxidase I (COI) mitochondrial gene. Genetic data on gastrotrichs are virtually non-existent (see Balsamo and Manicardi 1995), so this gene was chosen for analysis without prior knowledge of the amount of intra- and inter-specific variation in these species.

Materials and methods

Sandy sediments were collected from the intertidal of Petacciato Marina, Molise, Italy (41°11'N; 14°51'E), Norfolk, Virginia, USA (36°51'N; 76°43'W), and Pensacola Beach, Florida, USA (30°17'N; 87°08'W), in February, January and March 1995, respectively; they were kept at ambient temperature (20 °C), and were brought to the laboratory within one week. In the laboratory, specimens of *Xenotrichula intermedia* Remane were immediately extracted from the sediment by the narcotization–decantation technique (Pfannkuche and Thiel 1988) using 7% $MgCl_2$ aqueous solution, and were prepared for analysis. Morphological measurements were conducted on 15 living, relaxed specimens from each population. Individuals were transferred singly by glass micropipette to a slide, and were observed using either differential interference-contrast optics with a Microphot-FXA Nikon microscope, or phase-contrast using a Wild M 20 microscope. Several gastrotrichs from each location were photographed to serve as voucher specimens (Fig. 1). Measurements of 16 combined metric and meristic traits (Table 1) were obtained with an ocular micrometer, or from photographs. Comparisons among the three populations were performed using both univariate analysis (one-way ANOVA) and multivariate analysis (canonical discriminant analysis and principal-components analysis) in the Statistical Analysis System (SAS Version 6: SAS Institute Inc. 1990). Canonical discriminant analysis requires that individuals be preclassified into groups (here, "populations"), and is designed to reveal the linear combination of the original variables that maximizes the between-group variance. In contrast, principal-components analysis does not require any prior assumption about group membership, so it has the limitation that within-group variance is confounded with between-group variance.

For DNA analysis, 25 living specimens from each population were extracted, rapidly rinsed in deionized water, and transferred singly by either a micropipette or an Irwin loop to sterile 500 µl microfuge tubes containing 4 µl PCR buffer (Promega, Madison, Wisconsin). They were stored at −70 °C for future DNA amplification. Within 3 wk, the gastrotrichs were thawed, homogenized and boiled at 95 °C for 10 min to release DNA. For each specimen, 1 µl of the homogenate was transferred to a sterile 500 µl microfuge tube and mixed with 30 µl PCR reaction mix. Each 30 µl reaction consisted of 1 U Taq polymerase (Promega), 3 µl 10 × PCR buffer, 3 µl 25 m*M* $MgCl_2$ (both solutions supplied with the polymerase), 0.6 µl of each primer [1.25 m*M*, LCO1490 and HCO2198: Folmer et al. (1994); the numbers refer to the primer binding-locations relative to the *Drosophila yakuba* mtDNA sequence of Clary and Wolstenholme (1985)], 4.8 µl dNTPs (total 5 m*M*, New England Biolabs, Beverly, Massachusetts), and 18 µl sterile (autoclaved) distilled water. Reactions were amplified through 50 cycles in a Perkin-Elmer DNA thermal cycler using the following parameters: denaturation at 95 °C for 1 min, annealing at 40 °C for 1 min, extension at 72 °C for 1 min. Amplification was confirmed by electrophoresing 5 µl of each PCR product and appropriate negative controls in a 2% agarose gel containing 1 × tris-borate-EDTA buffer with a 1 kbase DNA size-ladder (GibcoBRL, Gaithersburg, Maryland). Gels were stained with ethidium bromide, and bands were visualized on a UV transilluminator. When additional DNA was needed, 1:100 dilutions in sterile water of the initial PCR product were used as a template in subsequent amplifications. PCR amplification of specimens from Florida was less successful than for other locations, and reamplification of the initial PCR product was also difficult for this material. The reasons for this difference are unknown.

Possible differences in the nucleotide sequence within and among populations were examined by digesting separate 8 to 24 µl aliquots of individual PCR products with 11 restriction enzymes having 4 to 6 base recognition-sequences (*Ase*I, *Ava*II, *Cfo*I, *Dde*I, *Hin*fI, *Mse*I, *Nla*III, *Rsa*I, *ScrF*I, *Taq*I, *Xba*I; protocol according to New England Biolabs, Beverly, Massachusetts). Restriction fragments were separated by electrophoresis in 15% acrylamide gels, and were visualized on a UV transilluminator as for the uncut PCR product. Fragment sizes were estimated by log-linear regression of mobilities relative to the DNA ladder (*Dra*I-digested lambda DNA, GibcoBRL). Preliminary comparisons between the banding pattern generated from whole versus purified PCR product (purifying kit,

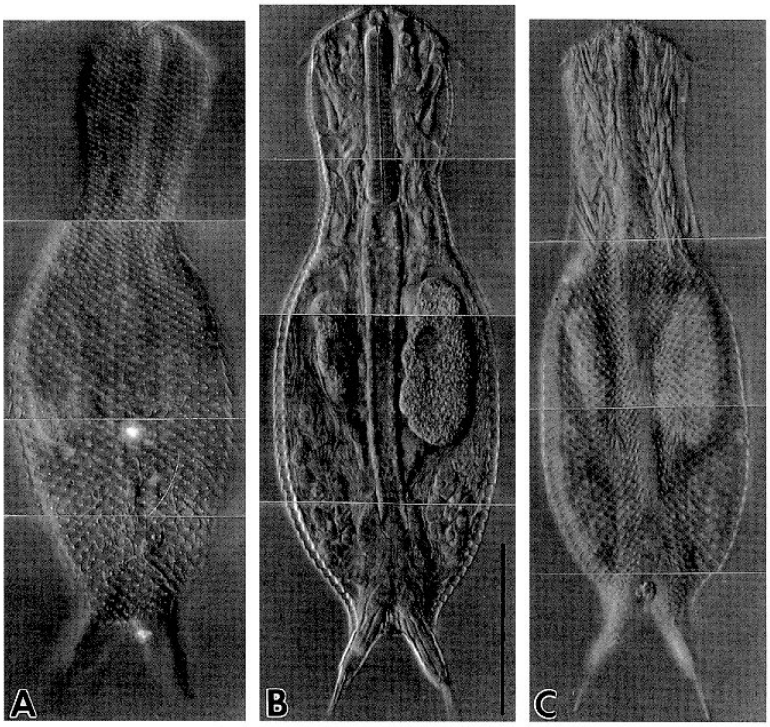

Fig. 1 *Xenotrichula intermedia* (specimen from Italian population). **A** dorsal view; **B** internal anatomy; **C** ventral view (*Scale bar* 50 μm)

Table 1 *Xenotrichula intermedia*. Summary statistics for 16 morphometric variables mean ± 1SD range in three populations (measurements in μm; $N = 15$ for each collection)

Variable	Italy	Virginia	Florida
	mean ± 1SD(range)	mean ± 1SD(range)	mean ± 1SD(range)
Total body length	203.5 ± 8.2 (188.0–219.0)	202.3 ± 4.4 (196.0–210.0)	203.0 ± 12.1 (185.0–228.0)
Pharynx length	52.0 ± 1.1 (48.0–59.6)	51.0 ± 2.0 (47.0–54.0)	45.6 ± 2.3 (45.0–52.0)
Furca length	34.7 ± 1.1 (31.6–36.0)	34.3 ± 1.1 (33.0–37.0)	34.6 ± 1.8 (29.6–38.0)
Adhesive tube length	14.8 ± 0.7 (13.7–19.0)	15.0 ± 0.6 (13.9–19.0)	15.7 ± 1.6 (13.6–18.0)
Pharynx anterior diameter	9.1 ± 1.0 (7.8–11.0)	10.0 ± 0.5 (8.5–11.0)	9.1 ± 0.9 (7.0–10.3)
Pharynx middle diameter	7.5 ± 0.5 (6.6–8.5)	8.5 ± 0.8 (6.0–9.2)	7.4 ± 0.7 (6.0–9.0)
Pharynx posterior diameter	9.9 ± 1.0 (7.8–11.1)	10.5 ± 0.5 (10.0–11.2)	10.0 ± 0.9 (8.2–11.2)
Head width	34.0 ± 2.4 (31.3–41.1)	33.2 ± 1.6 (30.8–36.0)	34.5 ± 3.7 (28.5–43.0)
Neck width	26.9 ± 1.0 (25.4–28.4)	27.9 ± 1.1 (26.0–29.4)	27.1 ± 2.7 (21.6–31.4)
Trunk width	48.4 ± 1.0 (47.0–50.0)	48.8 ± 1.5 (45.0–51.0)	52.4 ± 6.4 (41.1–70.6)
Base furca width	23.4 ± 1.3 (21.0–25.5)	23.4 ± 1.3 (22.0–27.0)	23.1 ± 1.4 (21.5–25.0)
Anus distance from indentation between furcal branches	24.7 ± 1.4 (22.2–28.0)	24.2 ± 0.6 (23.2–25.0)	24.1 ± 1.4 (20.8–26.0)
Mouth diameter	4.1 ± 0.1 (3.9–4.3)	4.1 ± 0.1 (4.0–4.3)	4.2 ± 0.4 (3.9–4.8)
No. of dorsal columns of scales	19.9 ± 1.0 (19.0–21.0)	20.1 ± 1.0 (19.0–21.0)	19.3 ± 1.3 (17.0–19.0)
No. of scales in dorsal median column	52.3 ± 1.4 (50.0–55.0)	51.5 ± 1.4 (49.0–54.0)	52.5 ± 1.1 (50.0–54.0)
No. of scales covering inner margin of furca	5.0 ± 0.0 (5.0–5.0)	5.0 ± 0.0 (5.0–5.0)	5.0 ± 0.0 (5.0–5.0)

[a] Differences among populations statistically significant at $P < 0.05$ (ANOVA)

Wizard™ PCR Preps System, from Promega, Madison, Wisconsin) yielded no differences, so we used mostly whole PCR products in our analysis.

Estimated sequence divergence at the nucleotide-site level (d), was calculated for each pairwise combination of haplotypes as per Nei (1987), using the shared-fragment approach (Nei and Li 1979). For any two haplotypes x and y, the proportion of shared fragments, F_{xy}, was estimated and then used to obtain the similarity coefficient G_{xy} by successive approximation. The following formula was then used to estimate d_{xy}:

$$d_{xy} = -(2/r)\log_e(G_{xy}),$$

where r = the average number of nucleotides per recognition sequence (here, 4.57).

Results

Fully mature (hermaphroditic) specimens of *Xenotrichula intermedia* ranged from 185 to 228 μm in total body length. The smallest and largest values were both found within the Florida population (Table 1). The furcal appendages varied from 29.6 to 38.0 μm in length, with extremes again among the Florida specimens. The longest adhesive tubes (19.0 μm) were among the Italian specimens, and the shortest (13.6 μm) among the Florida specimens. Pharynx length varied from 45.0 μm (Florida worms) to 59.2 μm (Italian worms). Regardless of the region of origin, the cuticular covering of the specimens consisted of typical pedunculated scales, arranged on the dorsal side in 17 to 21 columns containing 50 to 54 scales each. Univariate statistical analysis (ANOVA) performed on each of the above characters, as well as on 10 additional morphometric parameters (Table 1), could not detect significant differences among specimens of the three populations, except for the length of the pharynx. The pharynx was significantly ($P < 0.05$) shorter in the Florida specimens (mean = 48.7 μm) than either the Italian (52.3 μm) or Virginian (51.0 μm) specimens. Canonical discriminant analysis separated the Florida specimens from the others (Fig. 2), with pharynx length alone accounting for roughly half of the total discrimination (univariate $r^2 = 0.331$ for pharynx length versus multivariate $r^2 = 0.623$). In contrast to this result, principal-components analysis could not readily distinguish individuals from different locations (i.e., 10 principal components were required to explain 89% of the variance).

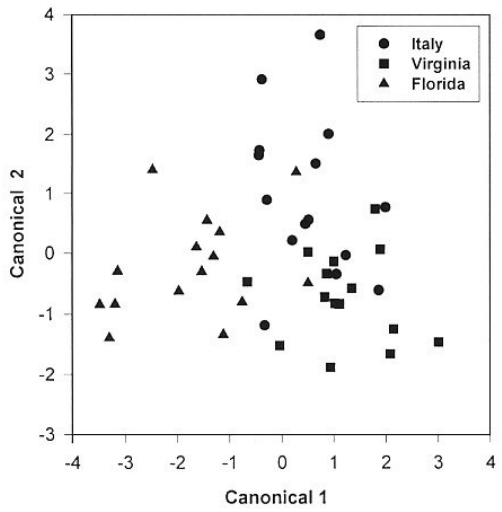

Fig. 2 *Xenotrichula intermedia.* Canonical discriminant analysis of morphological characters of specimens from three geographic areas. Florida specimens separated from others mainly by difference in pharynx length (see first paragraph of "Results")

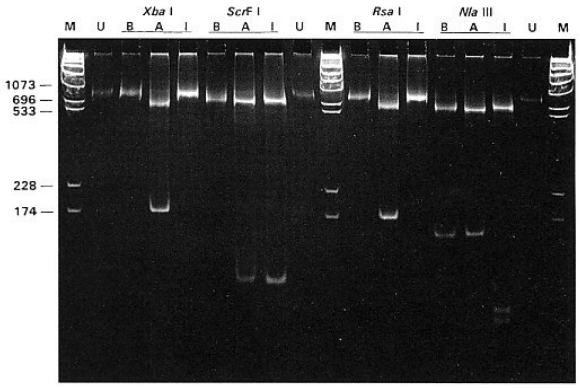

Fig. 3 *Xenotrichula intermedia.* Representative PCR–RFLP (polymerase chain-reaction–restriction-fragment length polymorphisms) banding patterns after separate digestion with *Xba*I, *Scr*FI, *Rsa*I and *Nla*III and electrophoresis in 15% acrylamide gels containing 1 × Tris-borate-EDTA buffer [*Numbers on ordinate* sizes of mobility standard, in base pairs; *U* amplified uncut PCR product; *M* marker (*Dra*I-digested lambda DNA); *B* Virginia B haplotype; *A* Virginia A haplotype; *I* Italian haplotype] Fragments of < 40 base pairs could not be visualized

The cytochrome oxidase I gene was successfully amplified from all 25 Italian specimens, 20 of 25 Virginian specimens, and 2 of 25 Florida specimens. Among the 11 endonucleases used in DNA digestions, one enzyme (*Mse*I) produced numerous small fragments that were difficult to size accurately (these were excluded from the analysis), and three enzymes (*Ava*II, *Cfo*I and *Taq*I) produced no cuts within the PCR product. Seven enzymes (*Ase*I, *Dde*I, *Hinf* I, *Nla*III, *Rsa*I, *Scr*FI and *Xba*I) produced informative restriction-fragment length polymorphisms revealing a high degree of variation among the specimens examined (Figs. 3 and 4 and Table 2). Each of the fragment-length profiles in Table 2 summed to a value that exceeded the size of the amplified product (710 base pairs). Similar discrepancies have also been reported in other PCR–RFLP studies, including some in which the estimated restriction-fragment size exceeded the size predicted from direct sequencing of the same product (Hrincevich and Foltz unpublished observations). These differences are probably due to the need for heavy loading of DNA in the experimental lanes for ethidium bromide visualization of small fragments. Incomplete digestion cannot account for the discrepancy, since within a profile different fragments were not related in size in a manner that would be consistent with partial digestion.

In total, four different fragment profiles (corresponding to an equal number of different mtDNA haplotypes) were detected. The Italian and the Florida

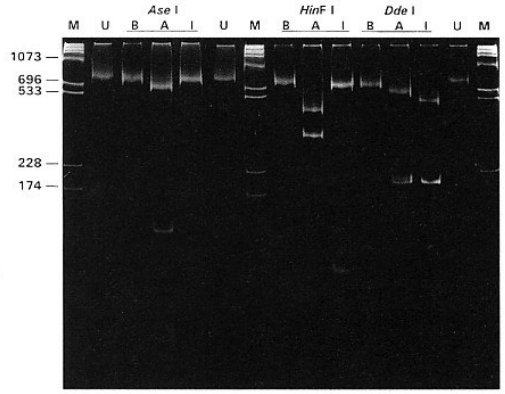

Fig. 4 *Xenotrichula intermedia*. Representative PCR–RFLP banding patterns after separate digestion with *Ase*I, *Hin*FI and *Dde*I. Further details in legend to Fig. 3

Table 2 *Xenotrichula intermedia*. Restriction-fragment profiles for three mtDNA haplotypes following separate digestion with indicated enzyme. Fragment sizes are shown in basepairs and have been rounded to nearest 5

Enzyme	Haplotype		
	I	Va	Vb
*Ase*I	740	665, 95	740
*Dde*I	595, 200, 50	665, 200	740, 50
*Hin*fI	730, 60	530, 375	765
*Nla*III	715, 50, 40	715, 130	715, 130
*Rsa*I	810	710, 165	810
*Scr*FI	755, 70	755, 70	755, 25, 20
*Xba*I	800	695, 165	800

populations each had a unique mtDNA haplotype (designated I and F, respectively), whereas two different mtDNA haplotypes (Va and Vb, with respective frequencies of 80 and 20%) were identified within the Virginia population. Overall, the seven informative restriction endonucleases produced 13, 14, and 11 restriction fragments in Haplotypes I, Va, and Vb, respectively (see Table 2 and Figs. 3 and 4 for details of fragment profiles). The number of shared fragments between the haplotypes ranged from 3 to 6 (Table 3), corresponding to estimated sequence divergences (*d*) of 5.3 to 11.5% (Table 4). Due to the small number of successful PCR products for Florida specimens (see second paragraph of "Results") and the fact that they were cut with a reduced set of restriction endonucleases compared to the other locations, the Florida specimens were excluded from the analyses shown in Tables 2 to 4. However, the estimated sequence divergences between the F haplotype and the other three (I, Va and Vb) were comparable in magnitude (5.7 to 8.3%) to those shown in Table 4.

Table 3 *Xenotrichula intermedia*. Number of shared fragments (*above diagonal*) and proportion of shared fragments (*below diagonal*) among three mtDNA haplotypes. Total number of restriction fragments for each haplotype is on diagonal line

Haplotype	I	Va	Vb
I	13	4	6
Va	0.296	14	3
Vb	0.500	0.240	11

Table 4 *Xenotrichula intermedia*. Nei's (1987) index of similarity (*above diagonal*) and estimated sequence divergence (*d*, *below diagonal*) among three mtDNA haplotypes

Haplotype	I	Va	Vb
I		0.802	0.885
Va	0.097		0.770
Vb	0.053	0.115	

Discussion

Specimens of *Xenotrichula intermedia* from the three areas (Mediterranean Sea, northwestern Atlantic, and northern Gulf of Mexico) looked remarkably alike. Morphological traits varied little both within and among populations. With the exception of pharynx length, differences among populations were statistically non-significant (Table 1), and only pharynx length was effective in separating one of the three populations using multivariate analysis. Because data were gathered using consistent methodology, our results reinforce the notion that morphologically similar taxa may inhabit distant geographic areas. Metric and meristic traits of our specimens fall well within the range of measurement reported in the literature for *X. intermedia* (Levi 1950; Gerlach 1953; Rao and Ganapati 1968; Luporini et al. 1973). However, when we compared our specimens with *X. intermedia* from Arcachon, France (Ruppert 1979), we found statistically significant differences in several major traits. Specifically, the means of body length, pharynx length, furca length and adhesive tube length of the French specimens were consistently smaller than the means of the specimens from each of the other populations. This last finding may be a morphological indication that cryptic species exist within the *X. intermedia* taxon (see later in "Discussion").

The second goal of our investigation was to determine the level of genetic variability in allopatric populations of cosmopolitan species. Our results show a very high degree of restriction-fragment length polymorphism among populations of *Xenotrichula intermedia*. Four mtDNA haplotypes were represented from three geographically distant areas. The estimated nucleotide sequence divergence for the COI gene

($d = 5.3\%$ to 11.5%), calculated for each pairwise combination of haplotypes, is quite high compared with previous studies of intraspecific mtDNA divergence, but consistent with estimates of mtDNA divergence from potential or confirmed interspecific comparisons. For the 16S rRNA gene, Bucklin et al. (1995) found that the level of mtDNA sequence variation within species of *Calanus* ranged from 0.3 to 2.6%, while the interspecific variation ranged from 7.3 to 23%. The most comparable study of COI divergence in terms of mtDNA region analyzed is that of Burton and Lee (1994; see also Burton 1994), who sequenced a 500-base-pair PCR product in the copepod *Tigriopus californicus* corresponding to mtDNA Positions 1756 to 2255 in *Drosophila yakuba* (Clary and Wolstenholme 1985). They reported sequence differences of 1 to 15.4% within single samples of *T. californicus*, and differences of up to 18% in allopatric (but nominally conspecific) samples. Other studies of COI sequence variation in marine invertebrates have used the COI-a and COI-f primers of Palumbi and Benzie (1991), which amplify Positions 2131 to 2811 of the *D. yakuba* sequence. For example, Knowlton et al. (1993) reported interspecific sequence-distance values of 6.4 to 20.4% in pairwise comparisons of snapping shrimp (*Alpheus* spp.) across the Isthmus of Panamá, and comparable interspecific COI-based distances were reported in horseshoe crabs by Avise et al. (1994).

The high nucleotide-sequence divergence found among *Xenotrichula intermedia* haplotypes raises questions regarding their affiliation with the same taxon, and suggests that the specimens with different haplotypes may in fact be distinct species. Because our specimens from different populations are generally morphologically indistinguishable, they may be considered sibling species (Mayr 1948). Recently, Knowlton (1993) reviewed the occurrence of marine sibling species and noted that, in comprehensive studies of single geographic regions, discovery of sibling species often results in four-fold or greater increase in diversity. Overall, one can expect the number of all marine species to increase by an order of magnitude if sibling species are considered. Thus, the discovery that populations of *X. intermedia* are actually sibling species falls well within Knowlton's prediction. Further study of specimens from other areas within the nominal range of *X. intermedia* may well reveal additional species within the complex, and more detailed examination of material from the Gulf of Mexico would also be worthwhile. The occurrence of sibling species at the same site (in our case at Norfolk, Virginia) should not come as a surprise, since the phenomenon has previously been reported in other taxa including meiobenthic forms (i.e., in the harpacticoid copepod genus *Tisbe*: Volkmann-Rocco 1972; Fava and Volkmann 1975), and planktonic forms (i.e., several species of *Calanus* with extensive overlapping geographical range in the north Atlantic Ocean: see Bucklin et al. 1995). Moreover, our results are in accordance with the prediction by Avise et al. (1987) that gross mtDNA genetic discontinuities in the absence of spatial separation are to be expected where reproductively isolated sibling species are inadvertently assayed as if belonging to a single species.

Several mechanisms of long-range meiofaunal dispersal have been suggested (e.g. meiofauna rafting on drifting materials or trapped in the ballast of sailing vessels; see also Gerlach 1977), and even plate tectonics has been suggested as a mechanism explaining amphi-Atlantic distribution (Sterrer 1973). Short-range dispersal via the water column associated with benthic storms could, over time, also lead to a long-range dispersal (Palmer 1988; Giere 1993). However, our results suggest that rates of dispersal among disjunct locations are very low and that extant regional populations exist as discrete units among which gene-flow appears to be negligible.

The marked phenotypic resemblance but genetic dissimilarity indicate that levels of morphological and molecular divergence are remarkably decoupled in the *Xenotrichula intermedia* species complex. Similar findings have been emphasized by evolutionary biologists working with other invertebrates and vertebrates (e.g. Cherry et al. 1978; Cunningham et al. 1992). Recently, large differences in mitochondrial DNA among morphologically similar species have been reported in penaeid shrimps (Palumbi and Benzie 1991) and in the "living fossil" horseshoe crabs (Avise et al. 1994). Two hypotheses could explain large genetic differences in phenotypically similar species: (1) the rate of molecular evolution might be rapid, or (2) the rate of morphological divergence might be slow. Because there are no data to substantiate the first hypothesis, Palumbi and Benzie (1991) contend that the phenomenon may be best explained by stabilizing selection acting on morphological or ecological characters while molecular differences accumulate at "typical" rates. Previous comparisons of morphological and molecular evolution in amphibians have in fact revealed similar patterns (Wallace et al. 1971). Such a scenario is probably also true among meiofauna, in which slow morphological divergence may be related to the relatively stable and uniform physiographic nature of their habitat. Interstitial animals, including gastrotrichs, are probably subject to selective pressure and stabilizing selection that favor distinctive phenotypic and perhaps physiological characters (as is shown by the convergence of morphological and life-history traits among unrelated meiobenthic groups) but may constrain morphological divergence when an adaptive peak is reached.

To our knowledge, this is the first study that has investigated the paradox of meiofauna cosmopolitanism using both morphological and molecular approaches. While morphology has been examined by previous investigations (see earlier in "Discussion"), molecular genetics is a relatively new field in meiobenthic studies. We are aware of only one published paper

(Litvaitis et al. 1994) dealing with this subject. Molecular genetic studies have never been carried out in the Gastrotricha, and evolutionary aspects of the COI mitochondrial gene (particularly within meiofaunal groups) are poorly known (but see Palumbi and Benzie 1991). Although there is evidence that this is one of the most conservative protein-coding genes in the mitochondrial genome (Brown 1985), we acknowledge that further studies are needed before making any conclusive statement as to what extent the COI gene can be used to infer phylogeny within "lower" metazoans such as gastrotrichs.

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