Molecular evidence for multiple species of *Oasisia* (Annelida: Siboglinidae) at eastern Pacific hydrothermal vents

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Introduction

Vestimentiferan tubeworms (Annelida: Siboglinidae) endemic to hydrothermal vents of the eastern Pacific are grouped into four monotypic genera (see Fig. 1 for distributions), *Ridgeia*, *Riftia*, *Tevnia*, and *Oasisia* (Black et al., 1997; 1998). However, previous investigators suspected the existence of multiple species in two of these genera. *Ridgeia* was thought to comprise several species (Jones, 1985; Tunnicliffe, 1988), but genetic studies revealed only a single species, *Ridgeia piscesae* Jones, 1985, with developmentally plastic tube morphology (Southward et al., 1995; Black et al., 1998). Similarly, differences in plume morphology led Jones (1985) to suspect that *Riftia* from the Gulf of California might be distinct from *Riftia pachyptila* Jones, 1981 on the East Pacific Rise (EPR) and Galapagos Rift (GAR). Again, genetic analyses revealed only a single species along the GAR and EPR from 27°N to 32°S (Black et al., 1994; Hurtado, 2002). Comparable morphological differentiation has not been noted for *Tevnia*, which also comprises a single genetically cohesive species, *Tevnia jerichonana* Jones, 1985, along the EPR from 13°N to 32°S (Hurtado, 2002). The genus *Oasisia* also has only one described species, *Oasisia alvinae* Jones, 1985 (Jones, 1985), but the existence of a second morphologically discrete species at 13°N on the EPR is suspected (Tunnicliffe, 1991).

The purpose of this study was to assess genetic variability of *Oasisia* throughout its distribution range. During the past 12 years, we have collected vestimentiferan samples from most of the known hydrothermal vent locations between Guaymas Basin (27°N) in the north to 32°S latitude along the EPR and on the GAR. *Oasisia* were obtained from four EPR localities in the sampled range: 21°N; 13°N; 9°N and 32°S. This study focuses on variation in DNA sequences of the mitochondrial Cytochrome Oxidase Subunit I gene (*mtCOI*) from these samples. Unlike the other three vestimentiferan vent genera of the eastern Pacific, *Oasisia* exhibits extraordinarily high levels of *mtCOI* diversity among sympatric and allopatric lineages. The present data are consistent with the existence of multiple species in this genus and warrant further studies of morphological and genetic differentiation to confirm whether these lineages indeed correspond to different species.

Material and methods

Oasisia specimens were collected using the submersible *Alvin*. Individuals were stored at -80°C prior to genetic analyses. For the present study, we examined 22 individuals from 21°N (dives 2233 and 3347), 11 from 13°N (dives 2227 and 2228), 15 from 9°N (dives 2351, 2356, 2359), and five from 32°S (dives 3338, 3339, 3341, and 3342) on the East Pacific Rise (Fig. 1).

We followed the manufacturer's protocol for the DNEASY kit (Qiagen, Inc., Valencia, CA) to extract total DNA from a vestimentum sample of each individual. An approximately 710-bp fragment of *mtCOI* was amplified using published primers and PCR conditions (Folmer et al., 1994). Both strands of each PCR product were sequenced on an ABI 377 automated sequencer (Perkin-Elmer/ABI, Foster City, CA). Sequences were proofread, aligned and assembled with Sequencher v. 4.1 (Gene Codes Corp., Ann Arbor, MI). Assembled sequences were truncated to 649-bp, a fragment that contained only clear and readable nucleotides.
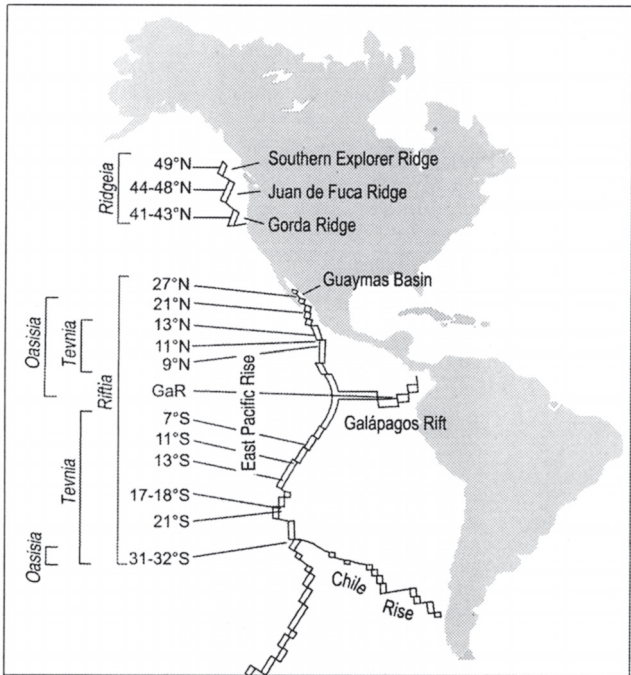
Figure 1. Map of known hydrothermal vent localities inhabited by tubeworms endemic to the eastern Pacific. Information based on published data (Black et al., 1998) and recent observations during an expedition to the southern East Pacific Rise. C. Fisher (pers. comm.) observed *Oasisia* at Mussel Bed on the Galapagos Spreading Center.

All phylogenetic analyses were performed with PAUP* v. 4.0b8 (Swofford, 1998). To infer evolutionary relationships among *Oasisia* haplotypes, we conducted maximum parsimony (MP), minimum evolution (ME), neighbor-joining (NJ), and maximum likelihood (ML) analyses. The MP and ME analyses were executed with heuristic searches using 50 stepwise random additions and TBR branch swapping. The MP analyses assumed equally weighted characters and equally weighted substitution types. The ME and NJ analyses assumed Kimura-2-parameter (K2P) distances between mitochondrial haplotypes (Kimura, 1980). To fit the best model for the ML analyses, we used the hierarchical Likelihood Ratio Test implemented by Modeltest 3.06 program (Posada & Crandall, 1998). Modeltest started with a NJ tree based on K2P distances and calculated likelihood scores for 56 different substitution models. The models are then compared to estimate the best model for the ML analyses of the data set. Bootstrap support values were calculated using MP and NJ analyses from a 50% majority-rule consensus tree, based on 1000 bootstrap replicates. We also included in our analyses published *mtCOI* sequences from *Ridgeia piscesae*, *Riftia pachyptila*, *Tevnia jerichonana*, *Escarpia spicata* Jones, 1985 and *Lamellibrachia barhami* Webb, 1969 (GenBank Accession nos. U74073, U74074, U74075, U74065, U74055). The trees were rooted with the same outgroups used by Black et al. (1997).

Results

We identified 25 distinct *mtCOI* haplotypes among the 53 specimens of *Oasisia* examined in this study. For *Oasisia*, 79 out of the 649 nucleotides scored for each individual were parsimony informative. All nucleotide substitutions were synonymous except one. The maximum nucleotide divergence (*d*) based on K2P distances among haplotypes was 10.1% (Table 1). All phylogenetic analyses revealed that *Oasisia* lineages form a well-supported monophyletic group with high bootstrap support (Fig. 2). *Ridgeia* appeared as *Oasisia*'s closest sister lineage and other vestimentiferans clustered outside the group containing *Ridgeia* and *Oasisia*.

Table 1. Average percent divergence based on Kimura-2-parameter distances within (diagonal) and between (above diagonal) *Oasisia* haplotypic groups.

	Haplotypic groups			
	I	II	III	IV
I	0.7	2.6	5.9	8.8
II		0.4	6.3	9.0
III			0.6	9.7
IV				0.2

Within the genus *Oasisia*, we identified four main groups among the 25 haplotypes (Fig. 2). All phylogenetic analyses revealed the same relationships among these four groups. Bootstrap support was high for the monophyly of each of these four groups, hereafter called haplotypic groups I through IV. Haplotypic group IV was the most divergent (d_{range} = 8.3–10.1%) among *Oasisia* groups (Table 1). Group IV comprised three minor variants ($\bar{d}$ = 0.2%) found exclusively at the 32°S locality. We define minor variants as the haplotypes observed within a haplotypic group. The remaining groups (I–III) were found only at northern localities. Among northern *Oasisia*, haplotypic group III was the most divergent (d_{range} = 5.3–6.9%) from groups I–II. Group III comprised two minor variants ($\bar{d}$ = 0.6%) found exclusively at 9°N (N = 5 individuals). Haplotypic groups I and II were the most closely related (d_{range} = 2.0–3.0%). Haplotypic group I comprised 13 variants ($\bar{d}$ = 0.7%) and group II comprised seven variants ($\bar{d}$ = 0.4%). Variants of group I were found in most of the individuals examined from 21°N (N = 19), but in few from 9°N (N = 2) and 13°N (N = 2). Conversely, variants of group II were found mainly among individuals examined from 9°N (N = 8) and 13°N (N = 9), but in few from 21°N (N = 3).

Discussion

The genus *Oasisia* exhibited the highest diversity for mitochondrial *COI* observed in any vestimentiferan genus. Sequence diversity was high in terms of the number of
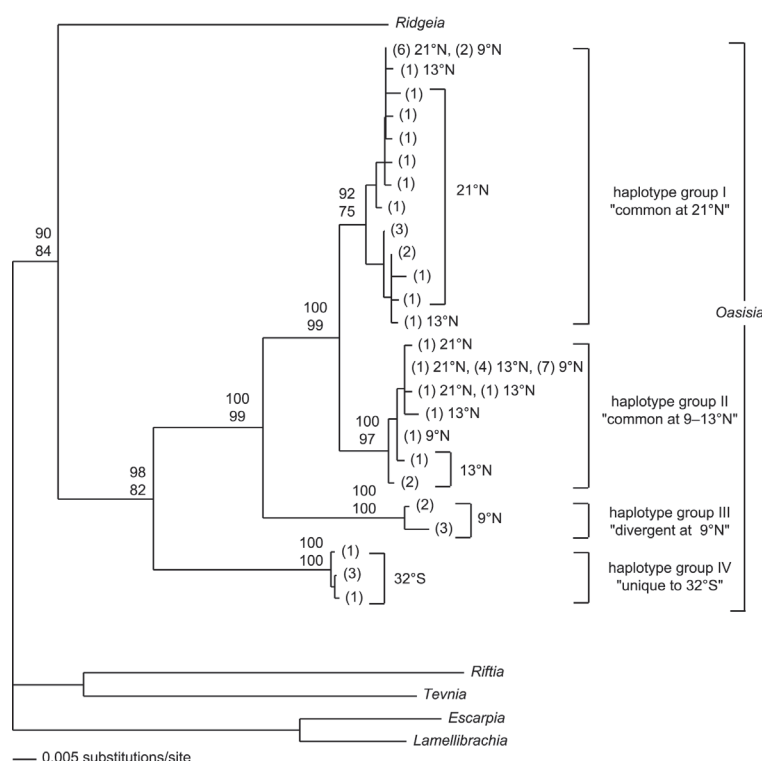
Figure 2. Neighbor-joining tree based on Kimura-2-parameter distances of the 25 *Oasisia* haplotypes and outgroup OTUs. Numbers next to nodes correspond to bootstrap values (above neighbor-joining and below maximum parsimony). Numbers in parenthesis correspond to the number of individuals per locality containing a particular haplotype. Although not shown, tree was rooted with the same outgroups used by Black et al. (1997).

haplotypes (25) and the degree of divergence (maximum = 10.1%) among them. Studies of *mtCOI* divergence in *Riftia* and *Tevnia* sampled from the same range in the eastern Pacific revealed much lower levels of divergence (Hurtado, 2002). For example, across more than 6500 km from Guaymas Basin to 32°S (Fig. 1), *Riftia pachyptila* exhibits a maximum divergence of only 0.15%. Similarly, across ~5000 km (13°N to 32°S), *Tevnia jerichonana* exhibits a maximum divergence of 1.3%. Maximum *mtCOI* divergence observed among populations of *Ridgeia piscesae* (41°N on Gorda Ridge to 49°N on Southern Explorer Ridge) is 0.8% (Black et al., 1998).

The cold-seep vestimentiferan genus *Lamellibrachia* provides a good example of a polytypic vestimentiferan genus. Maximum *mtCOI* divergence among populations of *L. barhami* in the North East Pacific (San Clemente Basin to Mendocino Fracture Zone) is only 0.3% (Hurtado, unpublished data). However, divergence between *L. barhami* and *L. columna* Southward, 1991, from the western Pacific is 5% (Black et al., 1997). Western Pacific *Lamellibrachia* comprise several divergent *mtCOI* lineages with a maximum sequence divergence of 1.1% between *L. columna* and *L.* sp. L1; however, reservations were expressed concerning the species status of these lineages (Kojima et al., 2001). It is notable, therefore, that the

divergences presently observed among some *Oasisia* lineages greatly exceed the values observed in *Lamellibrachia*.

The extraordinary level of *mtCOI* divergence among *Oasisia* lineages suggests that this genus comprises at least two distinct species and possibly more. Based on the present samples, the most divergent lineage, group IV, is limited to the region south of the Easter Microplate. The group IV lineage differed by 8.3–10.1% from other *Oasisia* lineages found north of the equator. Comparative data from other invertebrate species (reviewed by Knowlton, 1993) suggest that this level of divergence is likely to define distinct species. For example, 4–19% divergence distinguishes sister-species of snapping shrimp (Knowlton & Weight, 1998), 9.6% distinguishes species of penaeid shrimp (Palumbi & Benzie, 1991), 7.5% distinguishes named species of vesicomyid clams (Peek et al., 1997), and 1–6% distinguishes species of sea urchins (Lessios, 1981; Matsuoka & Hatanaka, 1991). However, mtDNA divergence alone does not demonstrate species-level separation. Studies of other genetic markers and morphological traits are needed to assess reproductive isolation and evolutionary independence of the *Oasisia* lineage at 32°S.

Northern *Oasisia* may also comprise more than one species. Divergence between groups I and II (2.0–3.0%) may or may not represent species-level differentiation. However, the observed frequencies of these groups differed dramatically between 21°N and 9–13°N localities. Group I was more frequent at 21°N and group II was more frequent at 9–13°N, suggesting that divergence among these lineages may be the result of geographical isolation. Notably, groups I and II differed greatly from group III (5.3–6.9%), which is more likely to correspond with species-level divergence. Group III was found sympatrically with groups I and II at 9°N. If multiple species exist at this locality, studies of nuclear genetic markers should reveal evidence for cryptic subdivision manifested as multilocus heterozygote deficiencies (Wahlund, 1928). Although sample sizes were very small in the allozyme study of Black et al. (1998), the data suggest such deficiencies might exist. Furthermore, morphological evidence suggests that more than one species of *Oasisia* may occur at northern EPR localities (Tunnicliffe, 1991; Schulze, 2001). Clearly, greater efforts should be made to obtain more comprehensive samples of *Oasisia* from northern EPR localities during future expeditions. Other genetic markers and morphology must be examined to assess the distinct nature of these lineages (work in progress).

Oasisia clearly has the highest *mtCOI* diversity of any vestimentiferan genus studied to date. What factors produced such diversity? Divergence of the putative *Oasisia* species found south of the Easter Microplate may be a consequence of geographic isolation alone. A large gap exists in the known

distribution of *Oasisia* along the EPR, ~3000 Km between 9°N and 32°S. Two other East Pacific Rise tubeworms *Tevnia jerichonana* and *Riftia pachyptila*, which do occur in this intermediate region, show limited divergence throughout this range (Hurtado, 2002). Observations of colonization and establishment at 9°N on the EPR suggest that *Oasisia* is not among the early successional species found at nascent hydrothermal vents, such as *Tevnia* and *Riftia* (Shank et al., 1998). Perhaps the rapid rate of spreading along most of the southern EPR (Haymon et al., 1991) causes a high frequency of habitat disturbance that prevents *Oasisia* from establishing thriving colonies in the region north of the Easter Microplate. Alternatively, *Oasisia* may have more limited dispersal ability than *Riftia* and *Tevnia*. Similarly, divergence between haplotypic groups I and II may also be the result of geographical separation between 21°N and 9–13°N. In contrast, divergence between haplotypic group III and haplotypic groups I–II may have occurred in sympatry in the northern EPR. Sympatric sibling species often exhibit distinct habitat preferences or some form of resource partitioning (Knowlton, 1993). Whether reproductive isolation and ecological divergence in terms of microhabitat differences and niche partitioning has occurred among these lineages at northern localities needs further examination.

Acknowledgements

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