Attributes of ribosomal DNA in alvinellid polychaetes from hydrothermal vents

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ABSTRACT

The ribosomal DNA (rDNA) of three alvinellid polychaetes, *Paralvinella palmiformis* Desbruyères & Laubier, *P. pandorae* Desbruyères & Laubier and *P. sulfincola* Desbruyères & Laubier, from deep-sea hydrothermal vents in the eastern Pacific Ocean, has been subjected to restriction-endonuclease digestion and the resulting restriction fragments compared with those of *Melinna palmata* Grube, a member of a closely-related polychaete family, the Ampharetidae. An interesting feature of the rDNA of representatives of both polychaete families was the tendency to cut with restriction enzymes containing an increased number of G-C (guanine-cytosine) bases in their recognition sequences. Increased GC-content is known to confer greater thermal and chemical resistance to the DNA molecule. This apparent similarity between the chemical composition of rDNA of representative alvinellids and *Melinna palmata*, a shallow-water species which tolerates highly-reduced and metal-rich sediments, suggests that this character may have arisen in the distant past primarily as an adaptation to chemically stressed environments.

RÉSUMÉ

Caractéristiques de l'ADN ribosomal de Polychètes Alvinellidés des sources hydrothermales

Les ADN ribosomaux (ADNr) de trois polychètes alvinellidés, *Paralvinella palmiformis* Desbruyères & Laubier, *P. pandorae* Desbruyères & Laubier et *P. sulfincola* Desbruyères & Laubier, provenant des sources hydrothermales profondes de l'est pacifique, ont été digérés par une endonucléase de restriction. Les fragments obtenus ont été comparés avec ceux de *Melinna palmata* Grube, une annélide polychète proche de la famille des alvinellidés, les ampharetidés. Un aspect important de l'ADNr des polychètes est leur tendance à se couper avec les enzymes de restriction contenant un nombre important de séquence de bases G-C (Guanine-Cytosine) dans leurs segments de reconnaissance. L'augmentation de la séquence en G-C est connue pour conférer à l'ADN une plus grande résistance thermique et chimique. Cette apparente similarité entre la composition chimique de l'ADNr représentatif des alvinellidés et *Melinna palmata*, une espèce d'eau peu profonde qui tolère les sédiments fortement réduits et chargés en métaux, suggèrent que ce caractère a dû se développer dans le passé comme une adaptation au stress chimique de l'environnement.

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INTRODUCTION

The polychaete family Alvinellidae is known only from hydrothermal vents in the Pacific Ocean (DESBRUYÈRES & LAUBIER, 1991). Two genera are described : *Alvinella* with two species and *Paralvinella* with nine species at vent sites on both sides of the Pacific. *Alvinella* species inhabit the sides of smokers on the East Pacific Rise only and they have substantial morphological alteration such as the insertion of bacterial filaments on the dorsum. The smoker habitat on Juan de Fuca Ridge and Marianas back-arc basin is occupied by *Paralvinella sulfincola* Desbruyères & Laubier and *Paralvinella hessleri* Desbruyères & Laubier, respectively. Other paralvinellid species live among vestimentiferan tubes or in sediments where the potential for high temperature encounters is low.

The alvinellid family has several characteristics reminiscent of the hypothetical ancestor of the terebellomorph order. The current model of systematic relationships among the alvinellid species is based on morphological analyses (DESBRUYÈRES & LAUBIER, 1991). *Alvinella* is the most derived genus. Within *Paralvinella*, there are four distinct morphological subgroups: *P. pandorae* is the most distinct and nearest the ancestral form while *P. palmiformis* and *P. sulfincola* belong to one subgroup marked by the form of the buccal tentacles. Although the latter two species share many features and are often collected together, genetic distance based on allozymes clearly differentiates the species (TUNNICLIFFE et al., in press).

This study examines three species from the Juan de Fuca Ridge: *Paralvinella pandorae pandorae* Desbruyères & Laubier, *P. palmiformis* Desbruyères & Laubier and *P. sulfincola* Desbruyères & Laubier. Habitat observations suggest that there is a preference for habitats with vent temperatures increasing in the order listed (Fig. 1). *P. p. pandorae* builds mucous sheaths in vestimentiferan clumps while *P. palmiformis* is sedentary but free-ranging in these lower temperature clumps and on sulphide chimneys formed by high-temperature fluids (TUNNICLIFFE *et al.*, 1985; DESBRUYÈRES & LAUBIER, 1986). *P. sulfincola* is only found on sulphide chimneys and, in the greatest numbers, within centimetres of superheated fluids (TUNNICLIFFE *et al.*, in press). Observations of the proximity of certain species to fluid flows in excess of 100 °C challenge current dogma that molecular stabilisation in metazoans requires temperatures less than 60 °C (BROCK, 1985).

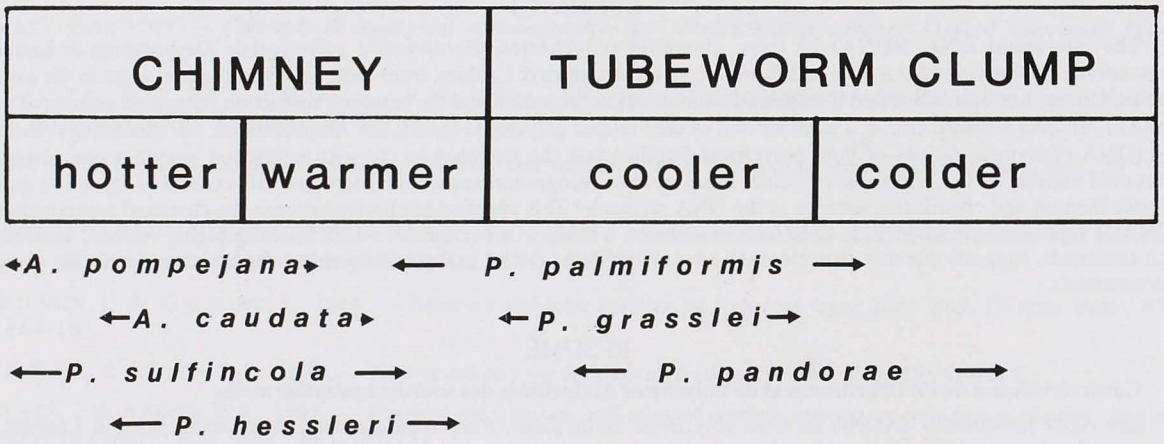

FIG. 1. — Approximate habitat ranges of alvinellid species. Hotter temperatures may range up to 65 °C as turbulent mixing carries blasts of hot water over the worms. There are no long-term records of chimney habitat temperatures to document actual ranges. Spot measurements and observations are the bases for this diagram. Little information is available for *P. hessleri*.

We recently reported the results of experiments which showed thermal stability of rDNA from a range of hydrothermal vent species (largely alvinellid polychaetes) was positively correlated with environmental temperature (DIXON *et al.*, 1992). This was attributed to a supposed increase in G-C (guanine-cytosine) content of the rDNA molecule. Increased G-C content confers increased thermal and chemical stability to the DNA molecule which is dependent on the number of hydrogen bonds: A-T (adenine-thymine) base pairs are linked by two bonds

whereas G-C pairs are linked by three (e.g. DARNELL *et al.*, 1986). This paper presents further evidence, based on restriction analysis results, of increased G-C levels in the rDNA of hydrothermal vent polychaetes and a close relative from highly-reduced and metal-rich, shallow-water environments, which suggests this character may have arisen in the distant past primarily as an adaptation to hostile chemical conditions.

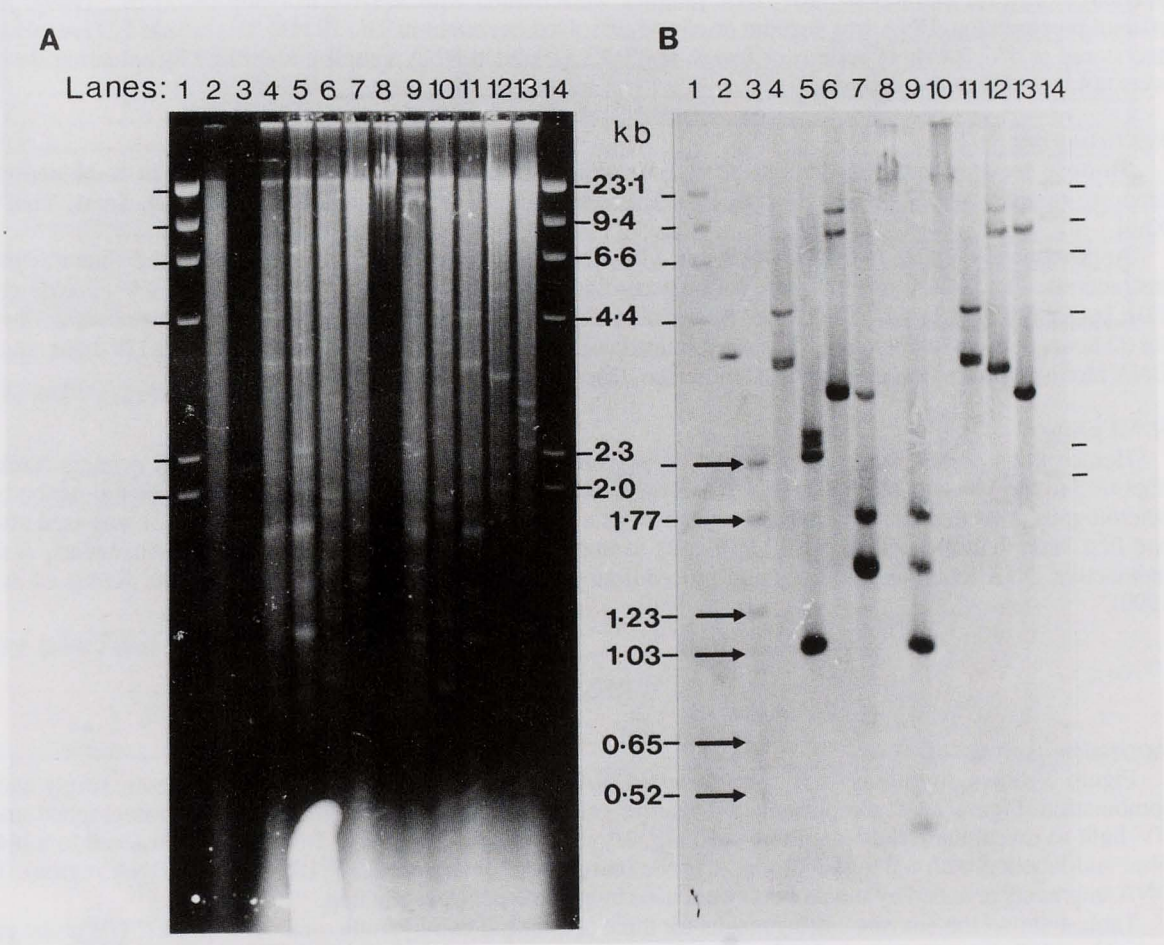

FIG. 2. — Hybridisation analysis of genomic DNA from a single *Paralvinella palmiformis*. (A) Photograph of 1% agarose gel after running at 30 volts for 16 h (0.1 volts cm^{-1} h^{-1}) and ethidium-bromide staining, showing numerous satellite bands. (B) After Southern transfer and hybridisation with the 2.9 kb pVW-PCR (*P. palmiformis* rDNA-) digoxygenin-labelled probe. Lanes 1 and 14, Lambda-HindIII DNA molecular-weight marker; Lane 2, SalI-digested *Mytilus edulis* genomic DNA (control) showing a characteristic 3.6 kb fragment; Lane 3, DNA molecular weight marker, pBR328 cleaved with BglI and HinfI; Lanes 4 - 13, *P. palmiformis* genomic DNA: Lane 4, EcoRI/HindIII double digest; Lane 5, BamHI/EcoRI double digest; Lane 6, SalI/HindIII double digest; Lane 7, SalI/BamHI double digest. Note, suspected partial; Lane 8, undigested DNA; Lane 9, SalI/EcoRI double digest; Lane 10, HindIII digest showing absence of cutting sites within the repeat unit (cf. also lanes 6 and 13); Lane 11, EcoRI-digest; Lane 12, BamHI-digest; and Lane 13, SalI-digest.

MATERIALS AND METHODS

Specimen collection

The three paralvinellid species were collected by submersibles on Juan de Fuca Ridge at two sites 100 km apart. Ten *P. palmiformis* specimens came from two vents on Axial Seamount (44°58.9' N, 130°13.3' W) and one vent on Cleft Segment (44°57.4'N, 130°13.8'W). The three *P. p. pandorae* specimens came from one vent at each site and *P. sulfincola*, two specimens, came from the base of a smoker at 44°58.9' N, 130°13.3' W. Another terebellomorph polychaete, the ampharetid *M. palmata* and the serpulid *Pomatoceros lamarckii* were collected subtidally at Plymouth, England; the dog-whelk *Nucella lapillus* and the mussel *Mytilus edulis* came from intertidal populations in southeast Cornwall.

Tissue handling and DNA extraction

High-molecular-weight DNA was purified by overnight digestion with proteinase K followed by repeated phenol/chloroform extractions. All DNA samples were treated with RNAse (SAMBROOK *et al.*, 1989). After ethanol precipitation, DNA was vacuum dried and dissolved overnight in TE (10 mM Tris, 1 mM EDTA, pH 8.0), and stored at 4°C for short periods or frozen at -20°C. Degraded DNA samples, identified by smearing on gels, were not included in the analysis.

Restriction analysis

Thirteen 6-cutter restriction endonucleases (Boehringer-Mannheim) were used singly and in combination on replicate samples of DNA : BamHI, Dra, EcoRI, EcoRV, HindIII, KpnI, PstI, PvuII, SacI, SalI, SmaI, XbaI and XhoI.

Approximately 2.5 mg of DNA was cut with restriction endonucleases according to the manufacturer's instructions. The DNA fragments were sorted according to size by gel electrophoresis using a 1 % agarose gel in TBE buffer (90 mM Tris-HCL, 0.89 mM boric acid, 2.5 mM EDTA, pH 8.3). Running conditions were 33 volts for 22 hours at room temperature. Gels were stained with ethidium bromide, photographed using UV-light, and the DNA blotted onto a nylon membrane (Genescreen, Dupont) (SOUTHERN, 1975).

rDNA probes

Homologous probes were manufactured by polymerase chain reaction (PCR), using PCR primers flanking regions 5 to the 18S and 28S genes (e.g. HOLLAND *et al.*, 1991), and cloned by conventional bacterial methods. A heterologous probe representing the entire rDNA repeat unit (pCtp 1550), (SCHMIDT *et al.*, 1982) was used after it had first been digested with HindIII and EcoRI to improve the labelling and hybridisation efficiencies. A non-radioactive DNA labelling and detection procedure was employed in this study (digoxygenin, KESSLER *et al.*, 1990).

RESULTS

Restriction analysis

Figure 2 shows digestions of *P. palmiformis* rDNA with a range of restriction endonucleases, singly and in combination. Figure 2A is a representative agarose gel stained with ethidium bromide and photographed under UV-light to reveal numerous satellite bands. Fig. 2B shows the same panel of DNA when transfered to a nylon filter and labelled with a 2.9 kb VW- PCR probe (based on *P. palmiformis*, 5' 18S - 5' 28S rDNA region). The DNA fragments revealed by the probe(s) were used to map the rDNA repeat unit.

Table 1 shows the enzyme cutting results for three coastal and two hydrothermal-vent species. The probe used was pCtp 1550 (SCHMIDT *et al.*, 1982) which has the potential to span the entire length of the repeat unit. Repeat unit length was estimated for the different species based on the combined lengths of the fragments produced by SalI digestion. In the case of *M. palmata*, which lacks a SalI in its repeat unit, the size was estimated from the combined fragment sizes produced by separate BamHI, EcoRI, SacI and PstI digests. *M. edulis* DNA was the only one which cut with all 13 enzymes. With the exception of *M. palmata*, the number of enzymes which cut was inversely related to the size of the repeat unit. This relationship held when the comparison was made based on the size of the large non- transcribed spacer (NTS), indicating that the number of enzymes cutting was not simply a function of sequence length. Based on the number of G and C bases in the recognition sequences of the enzymes

which cut within the rDNA, the rDNA of the two hydrothermal vent polychaetes appears GC-rich, i.e. has a higher G-C index, compared to the three shallow-water species (Table 1).

TABLE 1.— Recognition sequences of restriction endonucleases, G-C indices (on a scale of 0 - 6), and individual species scores: *Paralvinella palmiformis*, (*P. palm.*); *P.p .pandorae*, (*P. pand.*); *Melinna palmata*, (*M. palm.*); *Nucella lapillus* (*N. lap.*); and *Mytilus edulis*, (*M. edulis*).

Enzyme	Recognition sequence	G-C Index	*P. palm.*	*P. pand.*	*M. palm.*	*N. l ap.*	*M. edulis*
*Bam*HI	5' -G'GATCC- 3'	3	+	+	+	+	+
*Dra*I	5' -TTT'AAA- 3'	0	+	-	+	+	+
*Eco*RI	5' -G'AATTC- 3'	2	+	+	+	+	+
*Eco*RV	5' -GAT'ATC- 3'	2	-	-	-	+	+
*Hind*III	5' -A'AGCTT- 3'	2	-	+	-	+	+
*Kpn*I	5' -GGTAC'C- 3'	4	+	+	+	-	+
*Pst*I	5' -CTGCA'G- 3'	4	-	-	+	-	+
*Pvu*II	5' -CAG'CTG- 3'	4	+	+	+	+	+
*Sac*I	5' -GAGCT'C- 3'	4	+	+	+	+	+
*Sal*I	5' -G'TCGAC- 3'	4	+	+	-	+	+
*Sma*I	5' -CCC'GGG- 3'	6	+	+	-	+	+
*Xba*I	5' -T'CTAGA- 3'	2	-	-	-	+	+
*Xho*I	5' -C'TCGAG- 3'	4	+	+	-	+	+
Enzymes cutting within the repeat unit, n:			9	9	7	11	13
Mean G-C Index:			3.4	3.7	3.0	3.0	3.1
G-C index of those enzymes that did not cut within the repeat unit.			2.5	2.0	3.3	4.0	-

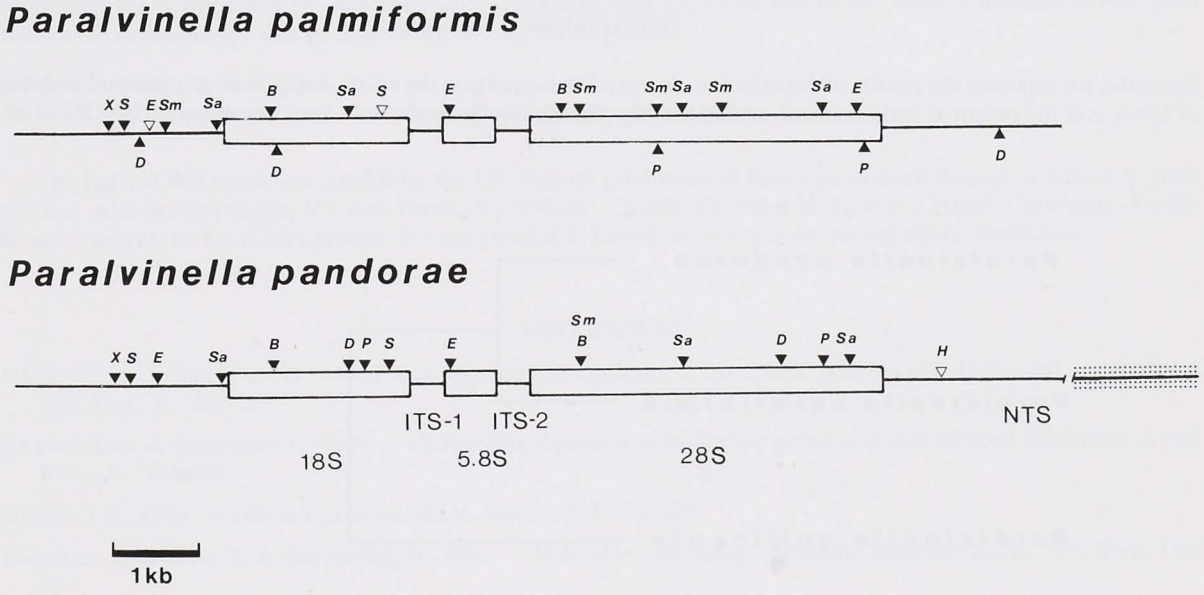

FIG. 3. — Restriction map of rDNA repeat unit of *Paralvinella palmiformis* and *P. pandorae*. Polymorphic restriction sites in *P. palmiformis* are indicated by open triangles. Restriction endonucleases which showed completely different cleavage patterns in the two species are shown below the restriction map of *P. palmiformis*. ITS-1 and ITS-2 = internal transcribed spacers; NTS = large non-transcribed spacer region; and 18S, 5.8S and 28S = the three rRNA-genes coding for the ribosomal subunits. B = BamHI; D = DraI; E = EcoRI; H = HindIII; P = PvuII; S = SalI; Sa = SacI; Sm = SmaI; and X = XhoI. The estimated 1.5 kb difference in repeat unit size was attributed to inter- specific variation in the size of the NTS region, indicated by the shaded portion in the map of *P. pandorae*. However, no enzyme was discovered which mapped to this region so confirmation must await the results of further investigations.

The three species of *Paralvinella* which were analysed for their rDNA structure had repeat unit lengths in the range 11 - 12.5 kb. This was significantly larger than the 7.2 - 7.6 kb values found for the three shallow-water species (one polychaete, two molluscs). The difference in repeat unit length between *P. pandorae* and *P. palmiformis* was traced to a duplication or some other sequence insertion affecting the NTS (Fig. 3). The significant differencein repeat unit length between *Melinna palmata* (7.5 kb) (Family Ampharetidae) and these three paralvinellids agrees with the conventional taxonomic separation of these two polychaete families.

Restriction mapping

Restriction sites for a total of eight enzymes were mapped for *P. palmiformis* and nine enzymes for *P. p. pandorae*; the extra one being HindIII (Fig. 3). These two species shared 10 restriction sites in common (37 %) out of a total of 27 mapped (Fig. 4). In contrast, *P. sulfincola* shared only four sites (22 %) in common with *P. palmiformis*, out of a total of 18 identified (DIXON & DIXON, unpublished), which may indicate a closer relationship between the former two species. (Note: insufficient high-quality DNA was extracted from the few specimens of *P. sulfincola* to carry out any detailed mapping of restriction sites.) Allowing for some heterogeneity between repeats and a degree of inter-individual restriction-site polymorphism, these data suggest that *P. palmiformis* is more closely related to *P. p. pandorae* than either is to *P. sulfincola*. Clearly, molecular adaptation to high temperature will have played an important part in speciation within this group. By way of contrast, the ampharetid *Melinna palmata* shared only six enzymes in common with the two paralvinellids (Table 1), and differed greatly in the number and size of fragments which these generated. The bulk of the variation in cleavage sites between *P. palmiformis* and *P. p. pandorae* mapped to genic regions within the rDNA repeat unit, although several polymorphisms were traced to the non-coding NTS. Lacking sequence information it is not possible to distinguish between introns and exons within genes. It seems highly probable that at least some of this intra-genic variation will involve "silent sequences", i.e. non-coding sequences which typically evolve rapidly (LI *et al.*, 1985).

DISCUSSION

Recently, we reported the results of experiments designed to investigate the relationship between thermal stability of DNA and the physical and chemical conditions experienced by hydrothermal vent organisms (DIXON *et al.*,

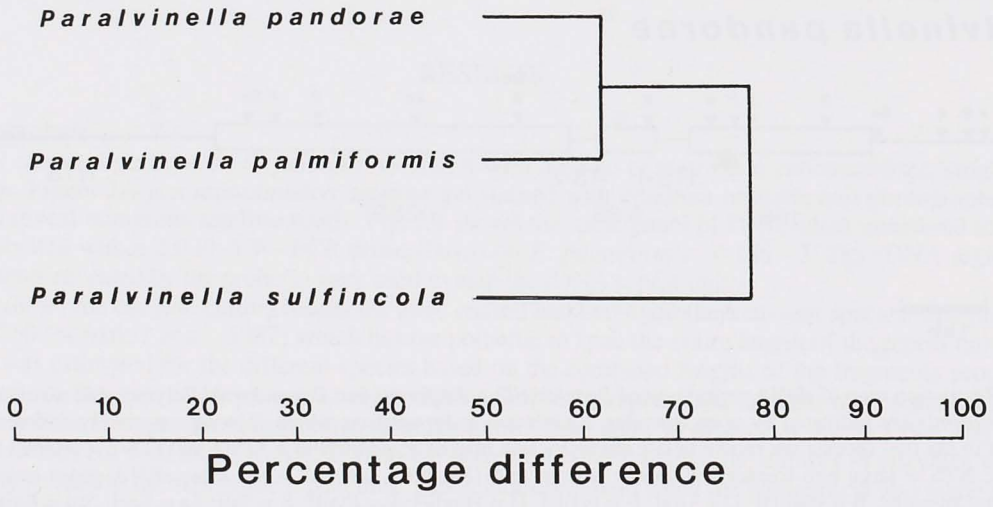

FIG. 4. — Unrooted tree based on percentage restriction-site differences between three species of *Paralvinella* from the Juan de Fuca Ridge.

1992). A positive correlation was found between the denaturing temperature of rDNA and environmental temperature for seven alvinellid species which was thought to be due to an increase in GC-content. In other organisms, increased GC-content has been shown to confer greater thermal and chemical stability to the DNA molecule (DARNELL *et al.*, 1986), and is an interesting molecular adaptation to hostile environmental conditions (e.g. BERNARDI & BERNARDI, 1990a, b). The results of the present investigation provide further evidence of increased GC-levels in the rDNA of some hydrothermal vent polychaetes and a shallow-water ampharetid, *M. palmata* (Table 1). The family Alvinellidae is closely related to, and perhaps derived from, the ampharetid polychaetes (DESBRUYÈRES & LAUBIER, 1986). The evidence presented here for increased GC-content in *M. palmata*, which tolerates highly-reduced and metal-rich sediments (GIBBS *et al.*, 1981), indicates that what may have arisen originally as an adaptation to harsh chemical conditions in shallow-water sediments, may have permitted colonisation of the high-temperature, and chemically hostile, hydrothermal vent environment in the distant past by a common ancestor (TUNNICLIFFE, 1992).

The topology of the unrooted tree shown in Figure 4 is divergent from present systematic relationships based on morphology which places *P. p. pandorae* as the most ancestral species of the genus. All other paralvinellid species have a different branchial form; among these species *P. palmiformis* and *P. sulfincola* form a sub-group with trifoliate tips on the buccal tentacles (DESBRUYÈRES & LAUBIER, 1991; TUNNICLIFFE *et al.*, 1993). A study of allozyme similarity among these species confirms the distance of *P. p. pandorae* but also makes a strong differentiation between *P. sulfincola* and *P. palmiformis* (D. JOLLIVET, pers. comm.). That morphological and molecular interpretations differ may be a function of the strong selection that is likely present on molecular functioning in an extreme habitat; morphological response may not be so strong. However, phylogenetic comparison of sequences that differ by more than 25 % is not recommended (UPHOLT, 1977); with greater differences there is increased probability that cleavage sites may be convergent (DOWLING *et al.*, 1990). The apparently high degree of divergence of these three species (up to 60 %) may reflect long evolutionary time, strong selection pressures, or both. While no certain fossil record is available, there are Cretaceous vent deposits with tubes reminiscent of those of Alvinella species (HAYMON & KOSKI, 1985); a large proportion of the vent fauna may be a Mesozoic relic (TUNNICLIFFE, 1992). High copy number sequences are generally not good candidates for constructing phylogenetic trees (HILLIS & MORITZ, 1990) and further work is required before good systematic relationships can be established among these species.

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