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Field distribution and sulphide tolerance of *Capitella capitata* (Annelida: Polychaeta) around shallow water hydrothermal vents off Milos (Aegean Sea). A new sibling species?

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Abstract The cosmopolitan polychaete *Capitella capitata*, known as a complex of opportunistic sibling species, usually dominates the macrobenthos of polluted or unpredictable environments. A population of *C. capitata*, termed *Capitella* sp. M, was found in a shallow water hydrothermal vent area south of Milos (Greece). Here, this population occurs close to vent outlets (termed the "transition zone"), an environment with steep gradients of temperature, salinity and pH and increased sulphide concentrations of up to 710 μ*M*. The field distribution of *C. capitata* in relation to sulphide concentrations around the vent outlets was investigated and sulphide tolerance experiments were conducted on laboratory-cultured worms to elucidate possible adaptations of *Capitella* sp. M to these extreme environmental conditions. In order to investigate whether the population from the Milos hydrothermal vent area can be considered a distinct sibling species within the *C. capitata* complex, crossbreeding experiments and analysis of general protein patterns were conducted with *Capitella* sp. M and three other *C. capitata* populations of different ecological ranges. *Capitella* sp. M showed high resistance (median survival time: 107 ± 38 h) to anoxia plus high sulphide concentrations of 740 μ*M*. It seems that the ability to survive high-sulphide conditions in combination with reduced interspecific competition enables the polychaete to maintain a continuous population in this rigorous habitat. From the extremely high tolerance to anoxia and sulphide, shown in both the crossbreeding experiments and the analysis of total proteins, it can be concluded that *Capitella* sp. M from the Milos hydrothermal vent area represents a separate sibling species within the *C. capitata* complex.

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Introduction

The sediment-dwelling polychaete *Capitella capitata* (Fabricius, 1780) has been found in almost every part of the world in shallow water areas of high and low latitudes, usually dominating the fauna of polluted or unpredictable environments (e.g. Grassle and Grassle 1974, references cited therein; Tsutsumi 1990; Pearson and Pearson 1991; Wu et al. 1991). To our present knowledge, this cosmopolitan species has never been described from shallow water or deep-sea hydrothermal vent areas. Only Kamenev et al. (1993) mentioned, without greater detail, the occurrence of Capitellidae around shallow hydrothermal vents in New Zealand.

For years it has been known that *Capitella capitata* consists of a complex of genetically distinct sibling species, which differ in their reproductive modes (Grassle and Grassle 1976; Grassle 1980), in the ultrastructure of eggs and ovarian follicle cells (Eckelbarger and Grassle 1983), and in genital spine, sperm and larval morphology (Eckelbarger and Grassle 1987). More species have been described in recent studies differing in developmental rates and dispersal pattern (Wu et al. 1991), in adult body size (Pearson and Pearson 1991), as well as in some ecophysiological features (Gamenick and Giere 1994).

In the present study field investigations were carried out on a shallow water hydrothermal vent area in the Aegean Sea (Milos, Greece) described by Dando et al. (1995b) and Thiermann et al. (1997), where a population of *Capitella capitata*, hereafter termed *Capitella* sp. M, was found in the vicinity of vent outlets. We analysed

the field distribution of this population in relation to sulphide concentrations and several other chemicophysical parameters in the sediments near the hydrothermal vent area. To elucidate possible adaptations of *C. capitata* to these extreme environmental conditions the resistance of *Capitella* sp. M to anoxia and sulphide was investigated in tolerance experiments with laboratory-cultured worms.

In order to investigate whether the population from the Milos hydrothermal vent area can be considered as a new sibling species within the *Capitella capitata* complex, morphological and biological characteristics of *Capitella* sp. M were investigated and crossbreeding experiments performed with three other *C. capitata* populations from different ecological ranges, i.e. the coastal North Atlantic (Grassle and Grassle 1976) and North Sea tidal flats (Gamenick and Giere 1994). In order to support the ecophysiological and biological analysis, we also compared the general protein patterns of *Capitella* sp. M with those of the other above-mentioned *Capitella* populations. Schmidt and Westheide (1994) and Westheide and Brockmeyer (1992) suggested this method as a useful tool for cryptic species differentiation in other annelid species complexes.

Materials and methods

Study area

The island of Milos is located on the Hellenic Volcanic Arc in the southern Aegean Sea. Field investigations were carried out in Paleohori Bay on the south coast of Milos, an area with numerous hydrothermal vents (Thiermann et al. 1994; Dando et al. 1995b). Vent fluids are characterized by a high concentration of carbon dioxide and a low content of methane, hydrogen sulphide and hydrogen (Dando et al. 1995a).

Samples were taken by SCUBA diving in the centre of Paleohori Bay in 10 m water depth. This area is characterized mainly by seagrass beds of *Cymodocea nodosa* (Ucria) Ascherson that partially surround a large area of bare sand (about 15 m in diameter). In the centre of this area white mats occur that consist mainly of silicates, elemental sulphur and bacteria (termed "white patch", Dando et al. 1995b). White patches are interspersed by hydrothermally active gas-venting outlets (Thiermann et al. 1997).

Field investigations

Two separate transects, each 5 m long and 4 m apart, were laid from the seagrass bed towards the white patch: Transect A was sampled in July 1993 (summer) and Transect B was sampled in October 1993 (fall) and in April 1994 (spring). Along the transects six sampling stations were positioned in 1-m intervals: Station 0 was located in the *Cymodocea nodosa* bed; Stations 1 and 2, in the border zone near the seagrass bed; Stations 3 and 4, in the transition zone close to the white patch with hydrothermal vents; and Station 5, within the white patch (Thiermann et al. 1997).

For sulphide, salinity and pH measurements porewater samples were taken (Howes et al. 1985) at each station along the transects from 2 and 5 cm sediment depth. In the laboratory total sulphide concentration (undissociated H_2S, HS^- and S^{2-}) was determined following the colourimetric methylene-blue method of Gilboa-Garber (1971), modified by Howarth et al. (1983). Salinity (‰) was measured with a hand refractometer and pH-values were recorded using an Ingold-Electrode connected to a pH-meter (Knick). Temperatures of the overlying water and at different sediment depths were measured in situ using sealed thermometers.

For faunal analysis three sediment cores were collected with Plexiglas tubes (inner diameter 5 cm) along the transects at each sampling station. Each core was subdivided into 0–2, 2–5 and 5–10 cm horizons to obtain the vertical distribution of the worms. The samples were fixed in buffered formaldehyde (final concentration 5 to 10 vol%), and sieved through a 500 μm mesh. Macrobenthic organisms were sorted and specimens of the polychaete *Capitella* sp. M were counted.

Laboratory studies

For laboratory experiments living specimens of *Capitella* sp. M were isolated from sediment samples collected from the border zone by gentle sieving through a 500 μm mesh. The individuals were kept in aquarium tanks (10 × 15 cm) containing a 3 to 5 cm layer of azoic (by freezing twice) natural sediment in aerated seawater (35‰ S) at room temperature. The cultures were fed with commercial fish food flakes (Tetramin). To ensure cultures were derived from the same population, juveniles hatching from single brood tubes were transferred to separate aquaria where they were isolated until used in experiments. In order to study the reproductive mode of *Capitella* sp. M, the cultures were checked every couple of days and new brood tubes were transferred to individual petri dishes. Here the hatching success, number and size of the larvae and juveniles were recorded under a dissection microscope.

For scanning electron microscope (SEM) analysis, specimens of *Capitella* sp. M were fixed in Trumps fixative, then rinsed with cacodylate buffer and exposed to ultrasonic treatment for 10 s to remove adhering debris. Subsequently, the worms were dehydrated in a gradient series of acetone, critical point dried and gold sputtered. For inspection a Cambridge Camscan DV 4 was used.

Tolerance experiments

The tolerance tests (six replicates) were carried out on adult *Capitella* sp. M cultured in the laboratory under normoxic conditions (aerated seawater) in experimental runs with the following treatments: (i) "normoxia (control)", artificial seawater (35‰ S) buffered with 10 m*M* HEPES (pH: 7.9 to 8.2) and aerated continuously; (ii) "anoxia", same seawater but percolated with pure nitrogen for 30 min prior to introduction of worms; (iii) "anoxia plus 160 μ*M* sulphide" and (iv) "anoxia plus 740 μ*M* sulphide", same seawater bubbled with nitrogen for 1 h with subsequent addition of a sulphide stock solution (made from $Na_2S \cdot 9H_2O$ crystals).

Ten worms were placed in a 60 ml jar (containing glass beads) for each treatment. Except for the control treatment, jars were tightly closed and placed into a 1 liter container filled with anoxic seawater at 16 °C and subjected to continuous nitrogen inflow in order to prevent oxidation of treatments. Worms were checked every 8 to 12 h under the dissecting microscope (without opening the jars) and dead worms counted. Mortality assessment was based on absence of tactile response after stirring the worms in their incubation jar and the loss of red hemoglobin colouration. Hence, "mortality" in these experiments combines both moribund and dead animals. In relation to the situation in the field, both conditions can be combined since both will lead to the worms being eaten or decomposing.

Oxygen content, sulphide concentration and pH of the incubation medium were determined at the beginning (after introducing worms into the jars) and at the end of each experiment; experimental sulphide and pH-conditions are given in Table 1. In Treatments ii, iii and iv oxygen concentrations, measured with a sulphide-insensitive polarographic electrode (Orbisphere), stayed below detection limit (1 μ*M* O_2), which, in this context, is considered as anoxia. For sulphide measurements the same colourimetric method as described for field studies was used. Over the experimental time sulphide concentrations in the jars decreased by less

Table 1 Mean (n = 6) pH-values and sulphide concentrations of the four treatments in the tolerance experiments

Treatment	pH-value	Sulphide (μM)
(i) Normoxia	7.55 ± 0.06	0
(ii) Anoxia	7.57 ± 0.09	0
(iii) Anoxia + 160 μM sulphide	7.58 ± 0.09	162 ± 31
(iv) Anoxia + 740 μM sulphide	7.59 ± 0.05	744 ± 44

than 15% of the initial value. The term "sulphide" used in this study includes undissociated H_2S, HS^- and S^{2-}.

The survival data were analysed using one-way ANOVA on the slopes of the lines obtained by regressing the survival rates in each experiment over time. Before running the analysis the assumption of homogeneity of variances was examined using Cochran's C-test (Winer 1971). Since one experiment in anoxic conditions with 800 μM sulphide was unsuccessful, the number of replicates per treatment was balanced by choosing at random five replicates from each of the remaining four treatments (Underwood 1981).

Crossbreeding experiments

Crossbreeding experiments were conducted including three other *Capitella capitata* populations cultured in the laboratory: *Capitella* sp. I (Grassle and Grassle 1976) from the North American east coast and *C. capitata* S and *C. capitata* L from North Sea intertidal mud flats (Gamenick and Giere 1994, in the present study termed *Capitella* sp. S and *Capitella* sp. L). Pairs of one male and one female in all 16 relevant combinations were kept in petri dishes (three replicates, overall 96 individuals) with little sediment at 33‰ S and 16 °C. Every week the pairs were checked and brood tubes were kept until hatching of larvae.

General protein pattern

The total banding protein pattern of *Capitella* sp. M was compared with that of *Capitella* sp. I, *Capitella* sp. S and *Capitella* sp. L. Some hours before starting the electrophoresis, live worms were removed from the sediment and placed into petri dishes filled with seawater for clearance of gut contents. Preferably single specimens (or up to four small specimens) were put into Eppendorf caps, and weighed before 30 to 60 µl distilled water was added in order to obtain an equivalent protein concentration in the extract. Samples were then homogenized with Potter pestles within the caps (on ice) and centrifugated for 10 min at 20 000 ×g.

Total proteins of *Capitella capitata* were separated by isoelectric focusing in 300-µm polyacrylamide gels Servalyt Precotes 125 × 125 mm with a range of 3 to 10 pH on a horizontal system (Sartophor). The applicator strip was positioned 2 cm from the cathode; 10 µl of supernatant was pipetted to 7 of 8 lanes. A control lane of Serva Protein Text Mixture 9 was applied in the eighth lane. Focusing was started at 200 V with a constant current of 7 mA for 30 min, and continued at a power of 4 W for about 120 min, until the voltage reached 1900 V. After focusing, the gels were fixed for 20 min in an aqueous solution (20 vol%) of trichloracetic acid.

General proteins were stained with Coomassie Violet R-150 (Serva Violet 17) 0.05% (w/v) in universal solvent (methanol:acetic acid:water, 40:10:50, v/v/v) for 10 min. Samples of two *Capitella capitata* populations were applied to each gel in order to obtain a direct pairwise-replicated comparison of banding patterns. The high reproducibility of this technique (tested with protein standard) allowed the comparison of protein patterns from different gels. Different numbers of electrophoretic migrations were performed for each *Capitella* population: *Capitella* sp. M, n = 12; *Capitella* sp. S, n = 11; *Capitella* sp. L, n = 10; *Capitella* sp. I, n = 11. The estimation of the pH-ranges was based on the Protein Text Mixture control lane.

A phenetic analysis of general protein patterns was done by the band-counting method (Backeljau et al. 1994). With each band considered as a distinct character, general protein patterns were compared pairwise. Ferguson's (1980) similarity index (S_F), based on the ratio between shared and unique bands, was used to compare the four *Capitella capitata* populations: $S_F = c/m$, where c is the number of shared bands and m is the maximum number of bands in one of the two compared profiles. Pairwise data of similarity index were included in a matrix (see Table 5). A dendrogram, constructed with the unweighted pair group method with arithmetic means (UPGMA) (Sneath and Sokal 1973), was used to visualize the level of differentiation among populations of *C. capitata*.

Results

Field investigations

Regardless of the season, the chemico-physical parameters followed the same pattern along Transects A and B (Fig. 1). In all cases temperature, salinity and sulphide concentrations increased and pH-values decreased markedly towards the white patch. Lowest sulphide

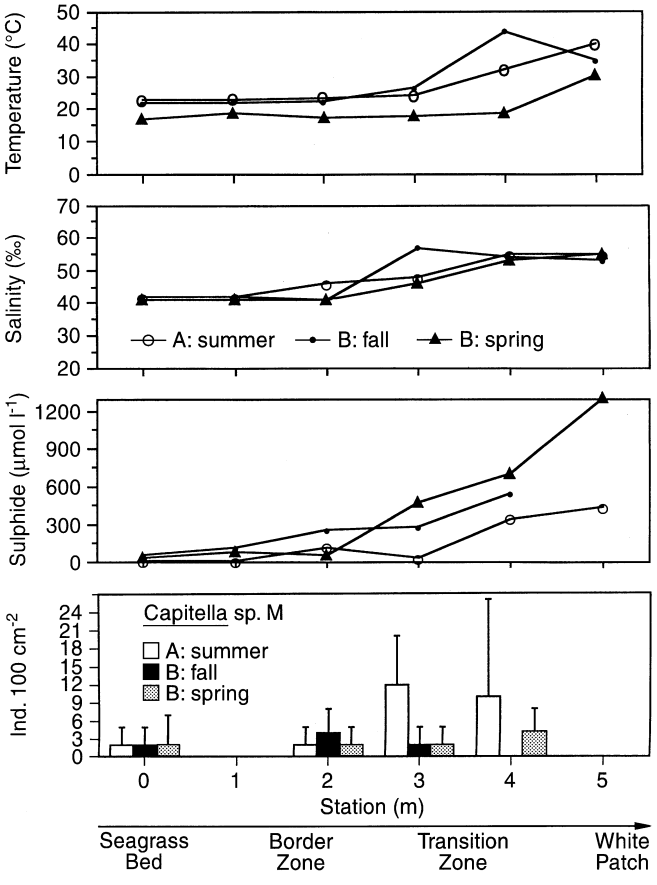

Fig. 1 *Capitella* sp. M. Chemico-physical parameters and mean abundance (n = 3) in 0 to 2 cm sediment depth along two 5-m transects, A and B in Paleohori Bay (Milos), in different seasons (*summer* July 1993; *fall* October 1993; *spring* April 1994)

concentrations varied between 2 and 88 μ*M* in the *Cymodocea* bed (Station 0) while high values were measured in the transition zone at 5 cm depth (Station 3, 1400 μ*M* sulphide, not shown) and in the white patch at 2 cm sediment depth (Station 5, 1310 μ*M* sulphide).

The distribution of *Capitella* sp. M among the stations was very distinctive, while both transects showed the same pattern. In general, the polychaetes occurred at low densities (2 to 12 ind/100 cm^2). However, along both transects their abundance increased towards the Stations 2, 3 and 4 close to the hydrothermal vents. Of all encountered *Capitella* sp. M, 82 ± 11% (n = 3) occurred in the upper 5 cm of this highly sulphidic area.

Laboratory studies

Features of adult and larval morphology and reproduction mode in *Capitella* sp. M are shown in Table 2 and documented in Fig. 2. Under laboratory conditions the wet weight of adults could range from 3.6 to maximal 9.2 mg, at a mean body length of about 2 cm. Females and hermaphrodites produced brood tubes, with eggs of a mean diameter of 228 ± 10 μm internally lining the walls. From each brood tube 100 to 300 free-swimming lecitotrophic trochophore larvae hatched (Fig. 2b). The barrel-shaped larvae had a mean length of about 460 μm (265 μm after SEM fixation) and possessed a well-developed prototroch and telotroch and a narrow-banded neurotroch. Under laboratory conditions, the larvae remained in a ''pelagic stage'' for several hours.

Table 2 *Capitella* sp. M. Features of morphology, life history and adult median survival rates (LT_{50}). Mean values ± SD (*after fixation for SEM)

Field occurrence:	
Depth distribution	down to 5 cm
Sulphide concentrations	up to 710 μ*M*
Adult morphology:	
Head (prostomium)	broad triangular
Tail (dorsal cleft)	absent
Teeth above fang	present
(Row/Formula)	(1/4)
Wet weight of worms	3.8 ± 2.2 mg (n = 45)
Sex	males, females, hermaphrodites
Eggs:	
Diameter	228 ± 10 μm (n = 30)
Eggs brood^{-1}	100–300
Larvae:	
Length	456 ± 8 μm (n = 30), 265 μm*
Proto-/Telotroch	well developed
Neurotroch	narrow band
Larval mode	lecitotrophic
Free swimming	several hours
Tolerance:	
LT_{50} anoxia	94 ± 20 h (n = 6)
LT_{50} anoxia + sulphide	107 ± 38 h (n = 6)

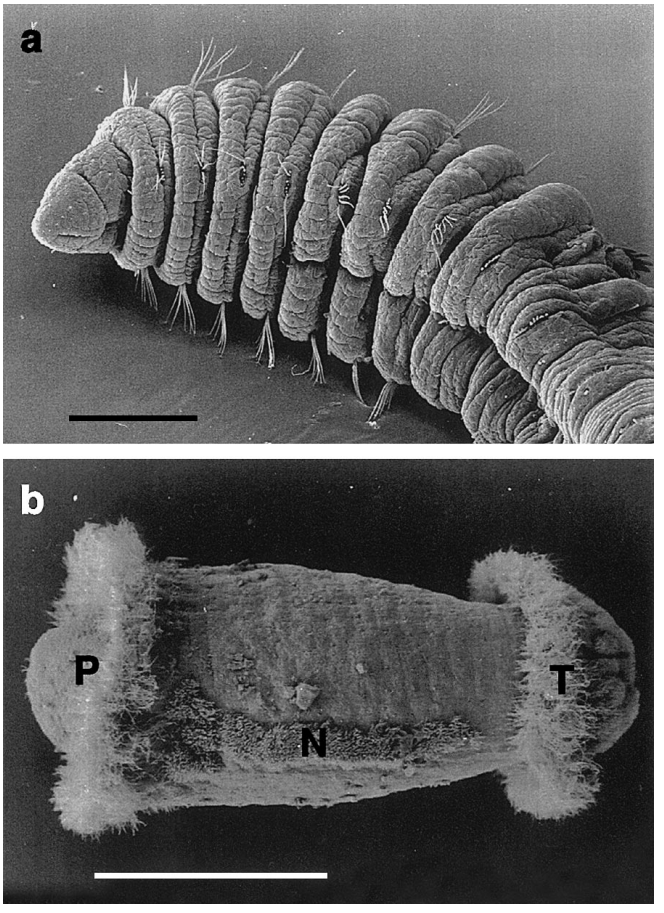

Fig. 2 *Capitella* sp. M. Scanning electron micrograph. **a** Anterior end of an adult female. *Scale bar* = 300 μm. **b** Newly hatched trochophore larvae. *Scale bar* = 100 μm (*P* prototroch; *N* neurotroch; *T* telotroch) (SEM by M. Mueller, Osnabrück)

Crossbreeding experiments

Interpopulation crossings with males and females from different *Capitella capitata* populations were unsuccessful in all replicates of the 12 possible combinations tested. Brood tubes and offspring were produced only in within-population crossings, here reproduction occurred in all replicates.

Tolerance experiments

It was surprising to record that under normoxic conditions without sulphide, specimens of *Capitella* sp. M died earlier (first mortality recorded after 20 h, median survival rate, LT_{50}: 80 ± 22 h) than under anoxia plus 740 μ*M* sulphide (first mortality after 50 h, LT_{50}: 107 ± 38 h; Figs. 3, 4). Compared to the normoxic control, anoxia and sulphide have no significant effect on the survival rate of *Capitella* sp. M (Table 3).

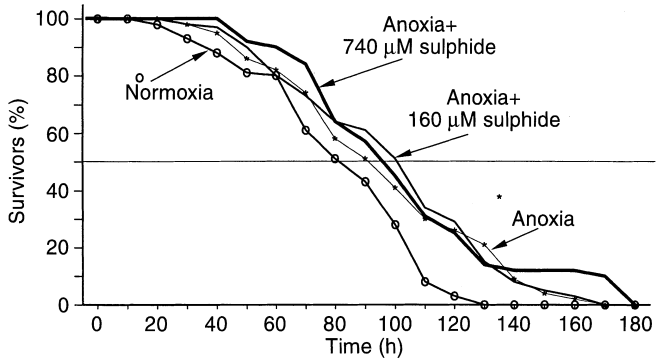

Fig. 3 *Capitella* sp. M. Percentage surviving four different treatments as a function of time (mean values of $n = 6$, SD not shown)

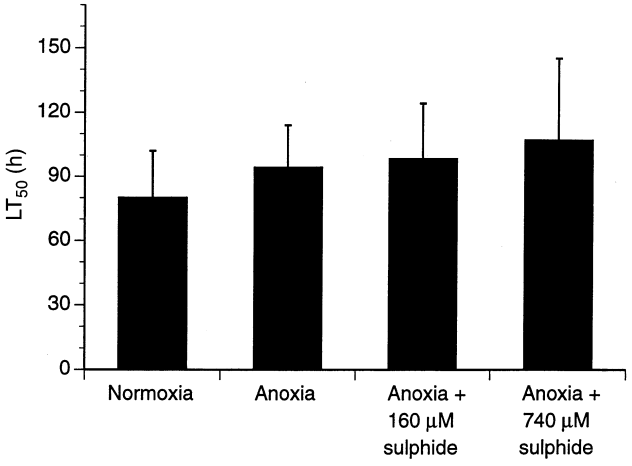

Fig. 4 *Capitella* sp. M. Mean ($n = 6$) LT_{50} rates for four different treatments

Table 3 *Capitella* sp. M. Statistical analysis of the effects of four different treatments on survival rates. Analysis performed on the angular coefficients of the regression lines of the survival as a function of time

Source of variation	*df*	MS	*F*	*P*	Cochran's *C*-test
Treatment	3	0.0290	0.6537	0.5922	0.4592 ($p > 0.05$)
Error	16	0.0444			

Protein patterns

The banding pattern of general proteins obtained by isoelectric focusing (IEF) was rather stable within each of the four investigated *Capitella capitata* populations. When comparing individuals of the same population, analysed on the same gel, the variability in the general protein pattern was extremely low, regardless of age and sex of the polychaetes. Small differences in the number of bands were observed between samples from the same population (Table 4). This could have been due to differences in the protein concentration of the sample or to the intensity of the staining, which may have led to a loss of the weaker bands. However, the low standard errors confirm the reproducibility of IEF.

Table 4 *Capitella capitata*. Mean number of bands detected in the general protein patterns of four populations (n number of replicates)

Population	*n*	Mean	SE
Capitella sp. I	11	22.0	1.15
Capitella sp. M	12	26.33	0.33
Capitella sp. L	10	27.33	0.88
Capitella sp. S	11	27.33	0.58

Table 5 *Capitella capitata*. Matrix of similarities among four populations based on Ferguson's similarity index (S_F)

Capitella	sp. I	sp. M	sp. L	sp.S
sp. I		0.66	0.67	
sp. M			0.75	
sp. L				0.86
sp. S				

Although the general protein patterns were similar between the specimens analysed, differences in the banding pattern allowed clear discrimination of the four *Capitella capitata* populations. In the pH-range of 8.3 to 4.4 approximately 20 bands were resolved. *Capitella* sp. M specimens shared on average 19 bands with the other *C. capitata* populations. The occurrence of several bands in the lowest pH-range of 4.4 to 3.5 was distinctive of *Capitella* sp. M; *Capitella* sp. S and *Capitella* sp. L showed the highest number of bands (Table 4), 25 of which they had in common. These two populations from the North Sea are characterized by the occurrence of a few marked bands at a pH > 8.0, which were absent in the other forms. Thus, the similarity index revealed that the two sympatric North Sea populations (*Capitella* sp. S and *Capitella* sp. L) showed the highest affinity (Table 5; Fig. 5). *Capitella* sp. M was closer to the two North Sea species than to *Capitella* sp. I. The Atlantic

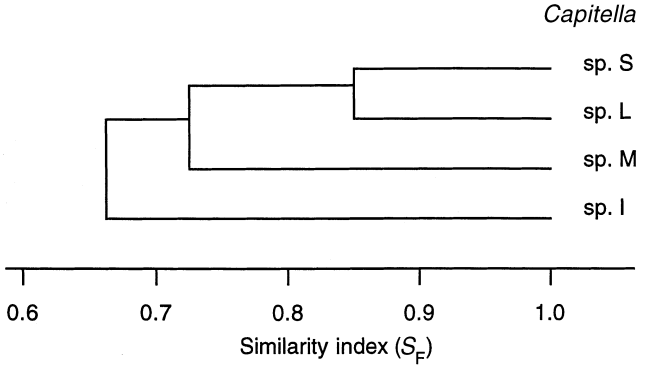

Fig. 5 *Capitella capitata*. Dendrogram showing the level of similarity among the four populations analysed. UPGMA cluster based on Ferguson's similarity index (S_F)

Capitella sp. I shared the lowest number of bands with the other populations (mean = 17) and exhibited some distinct bands in the cluster at pH 5.

The low variability of the intrapopulation banding pattern suggests that these data are useful for identification and differentiation of species, as it has been stressed by Schmidt and Westheide (1994) for polychaete species belonging to the genus *Nephtys*.

Discussion and conclusions

In the hydrothermal vent area of Paleohori Bay, *Capitella* sp. M had a clearly distinct distribution pattern. Along the transects from the seagrass beds into the hydrothermal area proper, it was the most abundant macrobenthic species in the border zone and in the transition zone close to the vents. Beside *Capitella* sp. M, only the gastropod *Cyclope neritea* (dominant species within the white patch) and the sulphide-tolerant nematode *Oncholaimus campylocercoides* were found that close to the vents (Thiermann et al. 1994, 1997). This distribution pattern of *Capitella* sp. M relates to other studies where *C. capitata* was described as the most opportunistic species indicative not only of organically enriched but also of unpredictable habitats (e.g. Grassle and Grassle 1974; Tsutsumi 1990; Pearson and Pearson 1991; Wu et al. 1991). The hydrothermal vent area can be characterized as an extremely unpredictable habitat, with high sulphide concentrations plus steep gradients of temperature, salinity and pH exerting an ecological stress on its inhabitants. *Capitella* sp. M can withstand these harsh conditions as corroborated by comparison of the tolerance data (see below).

Compared to other sibling species of the *Capitella capitata* complex (Grassle and Grassle 1976; Gamenick and Giere 1994) *Capitella* sp. M resembles in some morphological and life-history features *Capitella* spp. I and II from the North American east coast (Grassle and Grassle 1976). However, the lack of crosses with *Capitella* sp. I and its small size (only about one-fourth of the wet weight of *Capitella* sp. II), its somewhat different prostomium shape (broad triangular), and its larger, free-swimming larvae with narrow-banded neurotroches separate it from all the sibling species described so far.

In addition to the above features, the tolerance experiments on laboratory-cultured worms prove a genetically determined high resistance of *Capitella* sp. M to anoxia and high sulphide that is significantly different from other *Capitella* sibling species. In contrast to *Capitella* sp. S and *Capitella* sp. L from North Sea intertidal flats (Gamenick and Giere 1994), none of the anoxic and sulphidic test concentrations applied could significantly increase the mortality rate of *Capitella* sp. M. This indicates its high adaptation to the low-oxygen and high-sulphide conditions of the hydrothermal vent environment. Recent laboratory studies revealed that *Capitella* sp. M (LT_{50}: 107 ± 38 h) was even more tolerant to anoxic and sulphidic conditions than *Capitella* sp. I (LT_{50}: 65 ± 6 h, Gamenick 1997), which is known to inhabit organic-rich, highly sulphidic areas (Grassle and Grassle 1976; J.P. Grassle personal communication).

What might be the advantages for *Capitella* sp. M preferring a hazardous environment like the transition zone close to hydrothermal vents? According to Grassle and Grassle (1974), *C. capitata* can be considered a poor competitor. Therefore, a continuous population of *Capitella* sp. M would be likely to best thrive in such an area as the transition zone, with its highly reduced number of species (Thiermann et al. 1997). Another advantage of inhabiting the transition zone might be the slight enrichment of total organic carbon in this area (3.2% dry weight at the vent station, Dando et al. 1995b) compared to other regions in Paleohori Bay. On the other hand, the population of *Capitella* sp. M was never very large. Since in *C. capitata* population density is strongly influenced by food availability (e.g. Grassle and Grassle 1974; Tenore and Chesney 1985; Tsutsumi 1987; Qian and Chia 1992), this limited abundance in the hydrothermal field may be due to the comparatively scarce food in the area (compared to the "normal", eutrophic habitats of *C. capitata*). Judging from our tolerance experiments, restriction by high sulphide is of little relevance for this species. Adaptation to high sulphide concentrations can be acquired by different strategies. In most cases, sulphide was shown to increase the negative effects of hypoxia (Bagarinao 1992). The remarkable adaptation of *Capitella* sp. M to hydrothermal conditions is documented by the fact that, in contrast to most other animals, its survival rates under normoxic, anoxic and anoxic plus sulphide conditions do not differ significantly. Adaptation to sulphidic conditions always involves deep metabolic modifications. One of the most frequently chosen pathways in benthic invertebrates is the shift to an anaerobic metabolism (see reviews: Vismann 1991; Bagarinao 1992; Childress and Fisher 1992). A high capacity for long-term anaerobiosis might account for the similar tolerance response obtained in our experiments with *Capitella* sp. M under anoxia and anoxia plus sulphide. The same phenomenon was found in the brackish-water ostracod *Cyprideis torosa*, with its similar survival rates under conditions of hypoxia and hypoxia plus high sulphide of up to 1800 μM (Jahn et al. 1996; Gamenick et al. 1996).

In addition to its specific tolerance and the failure of crossings with other *Capitella* sibling species, the analysis of general protein patterns verifies the assumption that *Capitella* sp. M is genetically different from the other *Capitella* sibling species investigated here.

The IEF analysis showed that each of the four investigated *Capitella capitata* populations has a peculiar and unique protein-banding pattern, which is distinguishable by the identification of specific bands. Definite differences in the IEF profiles are extremely useful in the estimation of the overall distance between populations and the identification of species and subspecies (Tegelstrom et al. 1982; Solé-Cava and Levy 1987;

Schmidt and Westheide 1994), despite difficulties related to the genetic interpretations of the results (Levy et al. 1988). Congruence of all the reported results from ecological, physiological, morphological, biological and genetic investigations agree with the assignment of sibling species status to the *Capitella* sp. M population from the shallow water hydrothermal vents off Milos.

In conclusion, this paper offers the first evidence of a new sibling species within the *Capitella capitata* complex, occurring in the low organic but highly sulphidic environment of shallow water hydrothermal vents. *Capitella* sp. M is apparently intimately adapted to these specific conditions. The metabolic pathways involved may have been the driving force behind genetic differentiation, evidenced here by biological separation and different protein patterns, and resulting in physiological speciation.

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