

A MOLECULAR COMPARISON OF ALASKAN AND NORTH EAST ATLANTIC *HALICHONDRIA PANICEA* (PALLAS 1766) (PORIFERA: DEMOSPONGIAE) POPULATIONS

DIRK ERPENBECK*, ANN L. KNOWLTON**, SANDRA L. TALBOT***,
RAY C. HIGHSMITH** & ROB W.M. VAN SOEST*

*IBED/Zoological Museum, University of Amsterdam, P.O. Box 94766, 1090GT Amsterdam, The Netherlands

**Institute of Marine Science, University of Alaska Fairbanks, P.O. Box 757220, Fairbanks, Alaska 99775-7220, USA

***Alaska Science Center, U.S. Geological Survey, 1011 E. Tudor Road, Anchorage, Alaska 99503, USA

E-mail: erpenbeck@science.uva.nl

ABSTRACT

The intraspecific relationships between populations of Alaskan *Halichondria* cf. *panicea* are the subjects of ongoing research. In this study we compare CO1 sequences of Alaskan *Halichondria* cf. *panicea* with North East Atlantic *Halichondria panicea* and its sister species *Halichondria boverbanki*. Alaskan *Halichondria* cf. *panicea* form a well-supported sister group to the European *Halichondria panicea* / *H. boverbanki* species complex in the resulting gene tree and cluster distantly from their European conspecifics.

KEY WORDS

Halichondria, Southcentral Alaska, NE Atlantic, sponges, CO1, intrageneric diversity.

INTRODUCTION

The “bread-crumbs” sponge *Halichondria panicea* (Pallas, 1766) is one of the most common intertidal sponges of the Eastern Atlantic. In the sublittoral it is likewise abundant especially in northern areas, e.g., the coasts of the North Sea, and ranges down to more than 500m. In the intertidal region it occurs on upper, lateral and undersides of boulders and holdfasts of brown algae. *H. panicea* is one of the best-studied sponge species of the northern hemisphere. Substrate specificity, growth, biomass, production and energy budget (e.g. BARTHEL, 1986), faecal pellets (WOLFRATH & BARTHEL, 1989), rate of suspension feeding and energy costs (RIISGÅRD *et al.*, 1993) are documented as well as reproductive features (e.g. TOPSENT, 1911; HARTMAN, 1958; WAPSTRA & VAN SOEST, 1987; WITTE & BARTHEL, 1994). Associated fauna studied include alpha-proteobacteria (ALTHOFF *et al.*, 1998, ERPENBECK *et al.*, 2002), endosymbiotic nematodes (BONGERS, 1983), scallops (FORRESTER, 1979), annelids, crustaceans, pycnogonids, echinoderms and fish (FRITH, 1976). The distribution is the entire Northern Atlantic along both the European and American coast. Southward, the species reaches New England (DIAZ *et al.*, 1993) and the Mediterranean, although it is uncommon in the Mediterranean itself. High arctic occurrence has not been established with certainty, but the species

is certainly found in northern Norway, Iceland and northern Canada. Similar sponges occur in the North Pacific and their conspecificity with Atlantic populations is likely.

VETHAAK *et al.* (1982) studied ecology and distribution of *H. panicea* and its sympatric sister species *H. bowerbanki* Burton, 1930. Taxonomic separation of the two species rests primarily on the arrangement of the spicules in the dermal membrane (HARTMAN, 1958; GRAAT-KLEETON, 1965), and the size discrepancy in spicules from ectosome and dermis (VETHAAK *et al.*, 1982). Tolerance towards siltation is lower for *H. panicea* than for *H. bowerbanki* preferring somewhat more exposed habitats. However, the ecological range is broad and overlaps considerably with that of *H. bowerbanki*.

North Pacific (Alaskan) populations of *Halichondria* are currently studied to investigate their distribution, genetic diversity and population structure. These studies use independent molecular markers such as ITS and the mitochondrial cytochrome oxidase subunit 1 (CO1). The suitability of ITS in sponges on lower phylogenetic levels has been established in various studies (BORCHIPELLINI *et al.*, 2000), whereas the suitability of mitochondrial markers still has to be proven. WATKINS & BECKENBACH (1999) and WÖRHEIDE *et al.* (2000) analyzed in the to our knowledge first mitochondrial analyses of sponges the variability of the cytochrome oxidase subunit 2. To date only a rather small CO1 data set has been used for comparative sponge-associate phylogenies (ERPENBECK *et al.*, 2002), and population studies of *Crambe crambe* (DURAN *et al.*, 2002). In this study we compare phylogenetic trees based on CO1 sequences of the Alaskan *Halichondria* cf. *panicea* with *H. panicea* / *H. bowerbanki* sequences sampled in the North East Atlantic. Alaskan specimens conform to *Halichondria panicea* in morphological aspects, but as the clear distinction of NE Pacific *H. panicea* and *H. bowerbanki* needs further elaboration they will be here referred to as *Halichondria* cf. *panicea*.

MATERIAL AND METHODS

The NE Atlantic *Halichondria* spp. originated off the Brittany coast (France) and southern Netherlands. The *Halichondria bowerbanki* sequence was obtained from GENBANK (accession number AF437299). Alaskan sponge material was collected from different substrate types in Kachemak Bay, Alaska (see KNOWLTON *et al.*, 2002). One additional specimen of both *Hymeniacidon perlevis* (Halichondriidae) from the Netherlands and *Liosina paradoxa* (Dictyonellidae, out-group) from Indonesia were sequenced, three non-*Halichondria* sequences were taken from Genbank: *Amorphinopsis excavans* Carter, 1887 (AF437297), and a second sample of both, *Hymeniacidon perlevis* (Montagu, 1818) (AF437301) and *Liosina paradoxa* Thiele, 1899 (AF437303).

DNA fragments were amplified in a two-step PCR approach. A first PCR product was received using rather universal PCR primers under temperature regimes that were used in previous studies (ERPENBECK *et al.*, 2002). 1 µl of the PCR product was used as DNA template in a consecutive step in order to reamplify the sponge fragments specifically. For this step the following specific primers were designed: CO1porF1: 5' CCN CAN TTN KCN GMN AAA AAA CA 3' and CO1porR1 5' AAN TGN TGN GGR AAR AAN G 3' and used under a temperature regime of 3 min 94° C, followed by 35 cycles of (30 s 94° C, 30 s 45° C, 1 min 72° C) and a final elongation time of 10 min 72° C. All PCR products of the NE Atlantic sponges and some of the Alaskan products were excised from a 2 % TAE gel, re-extracted with Glassmilk in a 65° C water bath, washed three times with 80 % ethanol, dried

and resolved in 10 μ l H₂O before ligated in a pGEM T-easy vector (Promega) and subsequently cloned in *Escherichia coli* following the manufacturers protocol. Plasmid DNA was extracted in alkalic lysis following standard protocols (SAMBROOK *et al.*, 1989) and underwent cycle sequencing with labelled M13 primers. Both strands of the template were sequenced in a LiCor automated sequencer (LiCor). A partition of the Alaskan samples was sequenced directly using labelled and tailed primers. Sequences were controlled using the AlignIR software (LiCor), aligned and further processed with MacClade 4.03 (MADDISON & MADDISON, 1990). To verify their taxonomic origin all sequences were pasted into an enlarged CO1 taxon set in a procedure as discussed in ERPENBECK *et al.* (2002).

Phylogenetic analyses were based on parsimony, likelihood and distance methods. Tree reconstructions were performed using PAUP*4.0 (SWOFFORD, 2002). *A priori* to the maximum likelihood analysis we estimated the relative best fitting likelihood model using Modeltest 3.06 (POSADA & CRANDALL, 1998) under the Likelihood Ratio Test. Some 100 bootstrap replicates were performed for the maximum likelihood (ML) analyses under heuristic search, TBR branch swapping and addition sequence under 10 random replicates. Prior to the parsimony analyses the degree of saturation was estimated for the third codon position substitutions analogous to LEHMANN *et al.* (1998). The maximum parsimony (MP) bootstrap was performed with 1000 bootstrapping replicates under TBR branch swapping and addition sequence under 10 random replicates. Minimum evolution (ME) trees were reconstructed under the maximum likelihood distances and bootstrapped with 1000 replicates.

RESULTS

The 24 taxon data set (2 out-group, 22 in-group taxa) contained 462 characters. Modeltest estimated the HKY+G (HASEGAWA *et al.*, 1985) model as the relatively best fitting maximum likelihood (ML) model for our data set. A plot of the different substitution types against uncorrected “p” distances revealed a slight saturation of the TC-substitutions. They were excluded from the maximum parsimony (MP) analysis. All reconstruction methods show identical results (Fig. 1): *Halichondria* forms a monophyletic clade (MP: 85 % BP (bootstrap probability), ML: 68 % BP, ME: 79 % BP) in respect to the other Halichondriidae in the taxon set. The *Halichondria* clade is split up in a monophyletic NE Atlantic clade (MP: 100 % BP, ML: 100 % BP, ME: 100 % BP) and a monophyletic NE Pacific clade (MP: 98 % BP, ML: 68 % BP, ME: 97 % BP). In a further step we studied the relationships of Atlantic and Pacific congeners in an analysis using the closer related *Amorhinopsis excavans* (Halichondriidae) as out-group taxon. With this approach we prevented the loss of too much phylogenetic signal. The reduced *Halichondria* spp. / *A. excavans* taxon set contained 20 (1 out-group 19 in-group) taxa with 42 parsimony informative characters. Modeltest estimated the HKY (HASEGAWA *et al.*, 1985) model as the relatively best fitting maximum likelihood (ML) model for our data set. A plot of the different substitution types against uncorrected “p” distances revealed no saturated substitution type for the third codon position. The reduced taxon set harboured a stronger phylogenetic signal for the two geographical *Halichondria* clade, which lead to better support of the Alaskan *Halichondria* cf. *panicea* monophyly (MP: 99 % BP, ML: 90 % BP, ME: 98 % BP, *in brackets*). The intraspecific relationships between the Alaskan *Halichondria* are the subject of ongoing research and will not be discussed in this study.

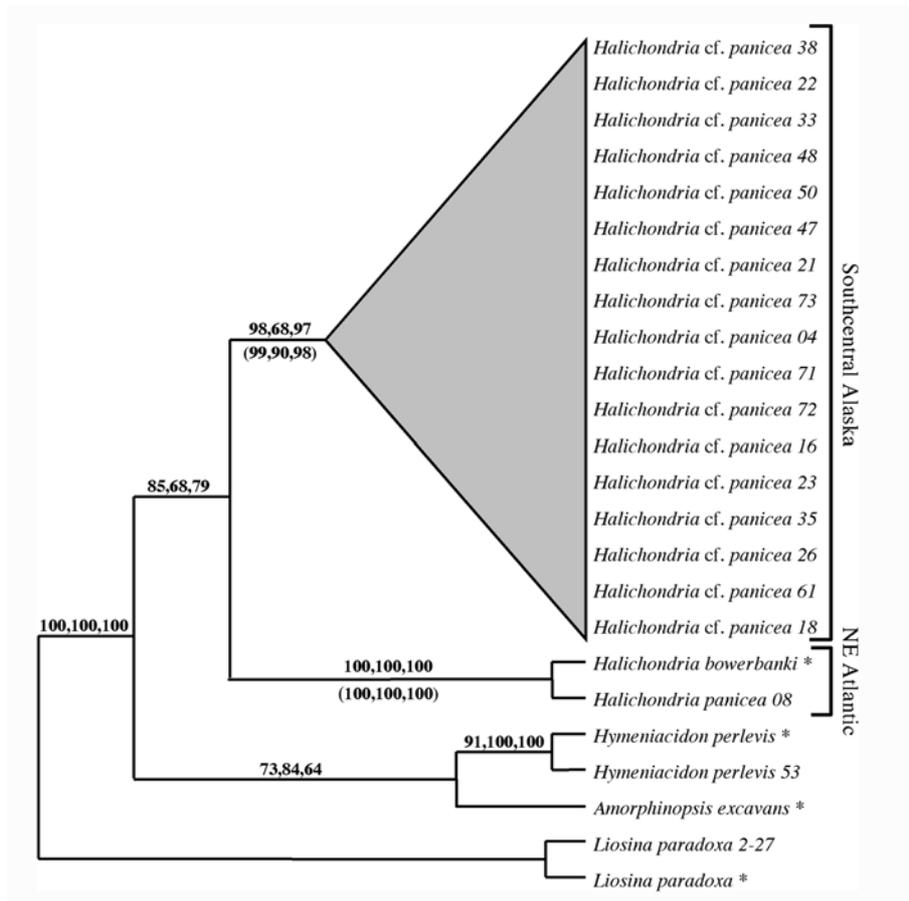


Fig. 1. Phylogenetic tree of analysed Alaskan and NE Atlantic *Halichondria*. The numbers above the branches indicate the bootstrap probability (BP) values for the MP (left), ML (middle) and ME (right) analyses. Numbers in brackets indicate the BP values for the reduced taxon set that consists only of *Halichondria* spp. and the outgroup taxon *A. excavans*. Numbers following taxon labels are internal sample identifications, asterisks denote sequences obtained from GenBank.

DISCUSSION AND CONCLUSIONS

The analyses reveal that the Alaskan *Halichondria* cf. *panicea* complex is genetically distant from the NE Atlantic *H. panicea* / *H. bowerbanki* species complex. The NE Atlantic specimens form a supported monophyletic clade and cluster as a sister group to all Pacific samples of *Halichondria* cf. *panicea*.

In the NE Atlantic *H. bowerbanki* and *H. panicea* are acknowledged valid species (VETHAAK *et al.*, 1982) although they share several overlapping taxonomic and ecologic features. VETHAAK *et al.* (1982) suggest from ecological studies that *H.*

panicea and *H. bowerbanki* originated from a parent North Atlantic species, out of which the East Atlantic populations evolved to *H. panicea*, while the West Atlantic populations evolved to *H. bowerbanki*. Invasion of each other's habitat would have taken place after the speciation event was completed. The outcome of our analysis could lead to various hypotheses about radiation of the *H. panicea* / *H. bowerbanki* species complex, but they should be used with great caution as the sample size of the Atlantic species is small and the analyses are based on a single gene. Furthermore, differences between NE Pacific *H. panicea* and *H. bowerbanki* are not as elaborately studied as for the NE Atlantic populations, but they differ clearly from other *Halichondria* congeners in the NE Pacific *i.e.* (cf. AUSTIN, 1985), *H. fibrosa* Fristedt, which possesses clearly longer oxeas, and the fistulose *H. (Eumastia) sitiens* (Schmidt). *H. lambei* Brøndsted [discussed to be “Lambe’s “rugose” form of *H. panicea*” by BRØNDSTED (1933) himself] is probably conspecific with *H. panicea*.

The results of this study could suggest that an additional ancestral line from the parental species invaded the Pacific Ocean, but it would need sample data of the North West Atlantic to verify. Alternatively the split between NE Atlantic and Alaskan *Halichondria panicea* might have taken place a long time ago and the Alaskan samples underwent multiple substitutions during their dispersal to the Pacific, exceeding the substitution rate of the Atlantic *H. panicea* and *H. bowerbanki*. Reticulate evolution, as yet undetected in sponges, cannot be excluded either as a cause for such interspecific patterns as those observed differences between CO1 sequences of NE Atlantic and Alaskan *Halichondria*.

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