

Final project report

<i>Project ID</i>	2004/01.04
<i>Title</i>	Antarctic marine protozoa and invertebrates: evolutionary biology, adaptive mechanisms and applicative perspectives
<i>Principal investigator</i>	Pierangelo Luporini
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<i>Duration</i>	Three years
<i>Assigned funding</i>	400.000,00 Euro

Activities and results

Material of study was basically represented by ciliates (Research Units headed by F. Dini at the University of Pisa, and P. Luporini at the University of Camerino), mollusks (RU headed by E. Olmo, Polytechnic University of the Marche Region), krill (RU headed by T. Patarnello, University of Padua), and tunicates (RU headed by V. Sbordoni, University of Roma 2). The unifying motif of the project was the study of the phylogenetic correlations linking Antarctic and non-Antarctic organisms, addressed to contribute new insight to the reconstruction of the evolutionary history of the Antarctic life. Nevertheless, each RU did as well pursue its own more specific research lines, including structural and functional studies of stress (heat-shock) genes, water-soluble psychrophilic proteins, homeotic genes, secondary metabolites, and genetic studies of intra- and inter-population polymorphisms. The following results appear to be of major general interest.

With regard to research on ciliates, most attention was focused on two species, *Euplotes focardii* and *E. nobilii*, of which in the laboratory there is a stable availability of a genetically significant variety of wild type strains and offspring clones. Of the former species, strictly psychrophilic and apparently endemic to the Antarctic coastal waters, it was cloned the hsp-70 heat-shock gene complex and shown how it behaves quite exceptionally due to a complete loss of the capacity of responding to thermal shocks, while in cells exposed to other environmental stresses such UV radiation and noxious chemicals it retains a very effective response. Of the latter species, which was found to inhabit the Arctic coastal waters in addition to the Antarctic ones, it was purified a family of water-borne signal proteins (usually designated as pheromones) of which it was then determined (in collaboration with the laboratory of Prof. K. Wuthrich at the ETH of Zurich) the specific structural modifications that are responsible for their biological activity at the freezing temperatures of the Antarctic, as well as the structure of the relative coding genes. *E. nobilii* was also used as excellent experimental system to prove, primarily on breeding and molecular grounds, that species of eukaryotic microbes have a bipolar (cosmopolitan) biogeographic distribution. Basically, it was proved that Antarctic and Arctic populations of this species can cross-fertilize and synthesize structurally homologous mating signal pheromones. In relation to studies on the Antarctic krill, it was cloned and fully characterized the phospho-gluco-isomerase gene from *Euphasia superba* and used, along with other more usual mitochondrial and nuclear marker genes, to obtain new insightful information on the genetic structure of a significant variety of populations of krill sampled from a sites of Ross sea, Antarctic Peninsula, South Georgia and South Shetland. Finally, research on mollusks was first directed to establish the phylogenetic relationships of the taxonomic groups represented by the chiton *Nuttallochiton mirandus*, the gastropod *Nebuccinum eatoni* and the bivalve *Adamussium colbecki*, and then, limitedly to *N. mirandus*, the complete set of the *Hox* and *ParaHox* genes (regulatory of the body ax development) was fully characterized and used to propose new implications about of the relationships of mollusks to the other Lophotrochozoa, as well as of the class Polyplacophora to the other classes of mollusks.

Products

A – papers in scientific magazines

1. **Papetti C, Zane L, Bortolotto E, et al**, 2005. Genetic differentiation and local temporal stability of population structure in the euphausiid *Meganyctiphanes norvegica*. *Marine Ecology, Progress Series*, 289: 225-235.
2. **La Terza A, Miceli C, Luporini P**, 2005. The gene for the heat shock protein 70 of *Euplotes focardii*, an Antarctic psychrophilic ciliate. *Antarctic Science*, 16: 23-28.
3. **Luporini P, Alimenti C, Ortenzi C, Vallesi A**, 2005. Ciliate mating types and their specific signal pheromones. *Acta Protozoologica*, 44: 89-101.
4. **Guidetti M, Marcato S, Chiantore M, et al**, 2006. Exchange between populations of *Adamussium colbecki* (Mollusca: Bivalvia) in the Ross Sea. *Antarctic Science*, 18: 645-653.
5. **Papetti C, Zane L, Patarnello T**, 2006. Isolation and characterization of microsatellite loci in the icefish *Chionodraco rastrospinosus* (Perciformes, Notothenioidea, Channichthyidae). *Molecular Ecology Notes*, 6: 207-209.
6. **Zane L, Marcato S, Bargelloni L, et al**, 2006. Demographic history and population structure of the Antarctic silverfish *Pleuragramma antarcticum*. *Molecular Ecology*, 15: 4499-4451.
7. **Odierna G, Aprea G, Barucca M, et al**, 2006. The karyology of the Antarctic whelk, *Neobuccinum eatoni* (Mollusca: Neogastropoda). *Italian Journal Zoology*, 73: 303-308.
8. **Odierna G, Aprea G, Barucca M, et al**, 2006. Karyology of Antarctic scallop *Adamussium colbecki*, with some comments on the karyological evolution of pectinids. *Genetica* 127: 341- 349.
9. **Barucca M, Biscotti MA, Olmo E, Canapa A**, 2006. All the three *ParaHox* genes are present in *Nuttallochiton mirandus* (Mollusca: Polyplacophora): evolutionary considerations. *Journal Experimental Zoology*, 306B: 164-167.
10. **Luporini P, Vallesi A, Alimenti C, Ortenzi C**, 2006. The cell-type specific signal proteins (pheromones) of protozoan ciliates. *Current Pharmacological Design*, 12: 3015-3024.
11. **Alimenti C, Ortenzi C, Vallesi A, Luporini P**, 2006. *Euplotes* pheromones. *Endocytobiosis Cell Research*, 17: 137-146.
12. **Papetti C, Lio P, Ruber L, et al**, 2007. Antarctic fish mitochondrial genomes lack ND6 gene. *Journal Molecular Evolution*, 65: 519-528.
13. **Papetti C, Susana E, La Mesa M, et al**, 2007. Microsatellite analysis reveals genetic differentiation between year-classes in the icefish *Chaenocephalus aceratus* at South Shetlands and Elephant Island. *Polar Biology*, 30: 1605-1613.
14. **Biscotti MA, Canapa A, Olmo E., Barucca M**, 2007. *Hox* genes in the Antarctic polyplacophoran *Nuttallochiton mirandus*. *Journal Experimental Zoology*, 308B: 507-513.
15. **Susana E, Papetti C, Barbisan F, et al**, 2007. Isolation and characterization of eight microsatellite loci in the icefish *Chaenocephalus aceratus* (Perciformes, Notothenioidei, Channichthyidae). *Molecular Ecology Notes*, 7: 791-793.
16. **Pedrini B, Placzek BJ, Koculi E, et al**, 2007. Cold-adaptation in sea-water-borne signal proteins: sequence and NMR structure of the pheromone En-6 from the Antarctic ciliate *Euplotes nobilii*. *Journal Molecular Biology*, 372: 277-288.
17. **La Terza A, Passini V, Barchetta S, Luporini P**, 2007. Adaptive evolution of the heat-shock response in the Antarctic psychrophilic ciliate, *Euplotes focardii*: hints from a comparative determination of the *hsp70* gene structure. *Antarctic Science*, 19: 239-244.
18. **Placzek WJ, Etezady-Esfarjani T, Herrmann T, et al**, 2007. Cold-adapted signal proteins: NMR structures of pheromones from the Antarctic ciliate *Euplotes nobilii*. *IUBMB Life* 59: 578-585.
19. **Negrisolio E, Bargelloni L, Patarnello T, et al**, 2008. Comparative and evolutionary genomics of globin genes in fish. *Methods Enzymology*, 436: 511-518.
20. **Vallesi A, Di Giuseppe G, Dini F, Luporini P**, 2008. Pheromone evolution in the protozoan ciliate, *Euplotes*: The ability to synthesize diffusible forms is ancestral and secondarily lost. *Molecular Phylogenetics Evolution*, 47: 439-442.
21. **Odierna G, Aprea G, Barucca M, et al**, 2008. Karyology of the Antarctic chiton *Nuttallochiton mirandus* (Thiele, 1906) (Mollusca: Polyplacophora) with some considerations on chromosome evolution in chitons. *Chromosome Research*, 16:899-906.
22. **Biscotti MA, Barucca M, Capriglione T, et al**, 2008. Molecular and cytogenetic characterization of repetitive DNA in the Antarctic polyplacophoran *Nuttallochiton mirandus*. *Chromosome Research*, 16: 907-916.
23. **Papetti C, Susana E, Patarnello T, et al**, 2009. Spatial and temporal boundaries to gene flow between *Chaenocephalus aceratus* populations at South Orkney and South Shetlands. *Marine Ecology, Progress Series*, 376: 269-281.
24. **La Terza A, Dobri N, Alimenti C, Vallesi A, Luporini P**, 2009. The water-borne protein signals (pheromones) of the Antarctic ciliated protozoan *Euplotes nobilii*: structure of the gene coding for the En-6 pheromone. *Canadian Journal Microbiology*, 55:57-62.

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25. **Vallesi A, Alimenti C, La Terza A, et al**, 2009. Characterization of the pheromone gene family of an Antarctic and Arctic protozoan ciliate, *Euplotes nobilii*. *Marine Genomics*, 2: 27-32.
26. **Alimenti C, Vallesi A, Pedrini B, et al**, 2009. [Molecular cold-adaptation: comparative analysis of two homologous families of psychrophilic and mesophilic signal proteins of the protozoan ciliate, *Euplotes*](#). *IUBMB Life* 61: 838-45.
27. **Vallesi A, Alimenti C, Di Giuseppe G, et al**, 2010. The water-born protein pheromones of the polar protozoan ciliate, *Euplotes nobilii*: coding genes and molecular structures. *Polar Science*, 4: 237-244.
28. **Guella G, Skopetra D, Di Giuseppe G, Dini F**, 2010. Structures, biological activity and phylogenetic relationships of terpenoids from marine ciliates of the genus *Euplotes*. *Marine Drugs*, 8: 2080-2116.
29. **Di Giuseppe G, Erra F, Alimenti C, et al**, 2010. Pheromone-mediated mating interactions and cross-fertilization between Antarctic and Arctic populations of the ciliate *Euplotes nobilii*. *Proceedings National Academy of Sciences of the USA*. In press.

B – book chapters

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C - proceedings of international conferences

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D – proceedings of national meetings and conferences

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E – thematic maps

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F – patents, prototypes and data bases

In specific relationship to the project, a multiplicity of new gene and protein sequences plus the three-dimensional NMR structures of four protein pheromones were originally determined. All the sequences and the NMR parameters that were object of publication (PhD theses included) have been officially deposited at EMBL Genbank Database, BMRB Data Bank and Swiss PDB, and their accession codes are available in the relative articles.

G – exhibits, organization of conferences, editing and similar

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H - formation (PhD thesis, research fellowships, etc.)

1. **Dott.ssa Chiara Papetti**, 2006. PhD Thesis in Biology. University of Padua. Title: *Population genetics and phylogeny of notothenioid fishes (Perciformes)*.
2. **Dott.ssa Maria Assunta Biscotti**, 2007: PhD Thesis in Biology and Marine ecology. Polytechnic University of the Marche Region. Title: *Molecular and cytogenetic characterization of repetitive DNA in species belonging to Mollusca phylum*.
3. **Dott. Valerio Passini**, 2007: PhD Thesis in "Environmental Science and Public Health". University of Camerino. Title: *Adaptive evolution of the heat shock response in Antarctic ciliates*.
4. **Dott.ssa Erica Bortolotto**, 2008. PhD Thesis in Biology. University of Padua. Title: *Molecular markers to analyze the genetic differentiation of krill populations*.
5. **Fellowship** (endorsed by the University of Padua, RU of T. Patarnello, 2006-08) on the theme "Time/space patterns of microsatellite DNA variability in Southern Ocean krill, *Euphausia superba*, and some of its notothenioid predators".

Research units

The following five research units were included in the project. The respective head and institutions are:

- (1) Prof. Fernando Dini (University of Pisa), f.dini@biologia.unipi.it;
 - (2) Prof. Pierangelo Luporini (University of Camerino), piero.luporini@unicam.it;
 - (3) Prof. Ettore Olmo (Polytechnic University of the Marche Region), etolmo@unplma.it;
 - (4) Prof. Tomaso Patarnello (University of Padua), tomaso.patarnello@unipd.it;
 - (5) Prof. Valerio Sbordoni (University of Roma 2), valerio.sbordoni@uniroma2.it
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Notes

In the perspective of supporting and opening new collaborations at international level, the leader of each Unit involved in the project is ready to provide samples of his Antarctic material to colleagues of foreign countries at the condition that this material is used only for basic research. Utilization of this material for applicative research is not excluded; however, a specific permission must be obtained from PNRA.