

Final project report

<i>Project ID</i>	2004/1.03
<i>Title</i>	Evolution and molecular adaptation of the oxygen-transport system in polar fish: Structure, function and genes of Hb - Erythrocyte viability - Erythropoiesis - Regulation of iron transport - Role of NO in circulatory-respiratory homeostasis - Cytogenetic characterization - Molecular phylogeny
<i>Principal investigator</i>	Ennio Cocca
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<i>Duration</i>	3 years
<i>Assigned funding</i>	260.000,00 Euro

Activities and results

Project 1.3/2004 proposed the investigation of various aspects of the molecular evolution and adaptations of the oxygen transport system in polar fish.

In the study of the structure and function of Hb from polar teleosts, the comparison of the properties of Hbs of fish living in Antarctic waters with those of species living in the Arctic or in a less extreme environment, as the sub-Antarctic, has represented a unique tool for understanding the molecular adaptations of these proteins.

The Hbs were investigated by analysis of primary structure, functional characterization (Bohr and Root effects), binding kinetics by laser flash photolysis and stopped-flow, Raman spectroscopy, study of aggregation process by light scattering. Very interesting results were the high conformational flexibility of *E. maclovinus* Hb1 and the aggregation process of Arctic Hbs.

The study of structural determinants has included the analysis of the Hb ferrous and ferric states, by EPR spectroscopy and by crystallography. The ferrous Hbs showed a change in quaternary structure similar to those identified for mammals Hb, while ferric forms showed quaternary structures intermediate between R and T. The possible functional activity of hemicrome, in particular as peroxidase and as superoxidase, has been investigated.

More structural and functional studies were carried out using chromatographic techniques, spectrophotometry and mass spectrometry. Oxygraphic methods were instead used to analyze the molecular interactions between Hb and red cell components.

Two polymorphisms in the Hb of *G. morua* have been identified through genetic studies and molecular modeling.

The 3D structures of polar skates Hbs were obtained by means of homology modeling.

The first characterization of the organization of globin genes in cartilaginous fish gave as results a geometry 3'-5', different from that found in teleost fish.

Several systems have been used to express mutants of Antarctic Hbs, in order to study the molecular basis of Root effect. The recombinant Hbs were expressed in high yields but in a non-soluble form.

The iron transport was investigated by the study of ferritin of Antarctic teleosts, characterized by an unusual structure, with higher flexibility that facilitates activity to low temperatures.

The study of gene expression of key proteins involved in oxidation-reduction, transport and acquisition of iron has been undertaken. By RT-PCR, 5'-RACE and Northern blot were studied genes of ceruloplasmin, transferrin, ferritin and DMT carrier protein.

The role of NO homeostasis in the circulation of Antarctic teleost has been studied by the analysis of NOS-NO-cGMP pathway in the heart of *C. hamatus*. It has been shown that the heart responds to the administration of endogenous and exogenous NO (analysis physio-pharmacological), presents active NOSs

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(NADPHdiaphorase and immunofluorescence), and releases NO in basal conditions (electrochemical analysis). Different isoforms of NOs were localized by immunohistochemistry.

Regarding the cytogenetic characterization of Nototenioidae, the research has involved the mapping of genes and chromosomal sequences of interest, and the analysis of karyotype diversification. Studies have focused on the 45S and 5S ribosomal genes, genes encoding the heavy chain of immunoglobulin (Ig), and transposable elements. Interesting results from the karyotypic diversification concerned the family Bovichtidae and the *E. maclovinus* species.

Products

A – papers in scientific magazines

1. Amelio D, Garofalo F, Pellegrino D, Giordano F, Tota B, Cerra MC (2006) Cardiac expression and distribution of nitric oxide synthases in the ventricle of the cold-adapted Antarctic teleosts, the hemoglobinless *Chionodraco hamatus* and the red-blooded *Trematomus bernacchii*. Nitric Oxide 15:190-8 IF 2,509
2. di Prisco G, Verde C (2006) Predicting the impacts of climate change on the evolutionary adaptations of polar fish. Rev Environ Sci Biotechnol 5: 309-321
3. Giordano D, Grassi L, Parisi E, Bargelloni L, di Prisco G, Verde C (2006) Embryonic beta-globin in the non-Antarctic notothenioid fish *Cottoperca gobio* (Bovichtidae) Polar Biol 30: 75-82 IF 1,502
4. Verde C, Giordano D, di Prisco G (2006) Molecular evolution of haemoglobins of polar fishes. Rev Environ Sci Biotechnol 5: 297-308
5. Verde C, Parisi E, di Prisco G (2006) Non-Antarctic primitive and modern notothenioid fish species: tracking the adaptive evolution in the structure, function and molecular phylogeny of hemoglobin. Deep-Sea Res 53: 1105-1114 IF 1,358
6. Ascenzi P, Bellelli A, Coletta M, Colosimo A, Falcioni G, Giacometti GM, Ippoliti R, Zolla L, Giardina B (2007) Multiple strategies for O₂ transport: from simplicity to complexity. IUBMB Life 59, 600-616 IF 2,857
7. De Rosa MC, Carelli Alinovi C, Galtieri A, Scatena R, Giardina B (2007) The plasma membrane of erythrocytes plays a fundamental role in the transport of oxygen, carbon dioxide and nitric oxide and in the maintenance of the reduced state of the heme iron. Gene 398, 162-171 IF 2,871
8. De Rosa MC, Carelli Alinovi C, Russo A, Giardina B (2007) Binding modes of L35 to alpha- and beta-semihemoglobins: structural insights into the inequivalence of alpha- and beta-subunits of hemoglobin. Biochem Biophys Res Co 354(3):720-6 Epub Jan 17 IF 2,749
9. di Prisco G, Eastman JT, Giordano D, Parisi E, Verde C (2007) Biogeography and adaptation of notothenioid fish: hemoglobin function and globin-gene evolution. Gene 398: 143-155 IF 2,871
10. Ghigliotti L, Mazzei F, Ozouff-Costaz C, Bonillo C, Williams R, Cheng C-H, Pisano E (2007) The two giant sister species of the Southern Ocean, *Dissostichus eleginoides* and *Dissostichus mawsoni*, differ in karyotype and chromosomal pattern of ribosomal RNA genes. Polar Biology 30: 625-634 IF 1,734
11. Giordano D, Parrilli E, Detta A, Russo R, Barbiero G, Marino G, Lecointre G, di Prisco G, Tutino L, Verde C (2007) The truncated hemoglobins in the Antarctic psychrophilic bacterium *Pseudoalteromonas haloplanktis* TAC125. Gene 398: 69-77 IF 2,871
12. Giordano D, Vergara A, Lee H-C, Peisach J, Balestrieri M, Mazzarella L, Parisi E, di Prisco G, Verde C (2007) Hemoglobin structure/function and globin-gene evolution in the Arctic fish *Liparis tunicatus*. Gene 406: 58-68 IF 2,871
13. Manconi B, Olianas A, Sanna MT, Messana I, Demurtas L, Castagnola M, Giardina B, Pellegrini M (2007) Functional characterization of the single hemoglobin of the migratory bird *Ciconia ciconia*. Comp Biochem Physiol B Biochem Mol Biol 147, 242-249 IF 1.651
14. Marino K, Boschetto L, de Pascale D, Cocca E (2007) Organisation of the Hb1 genes of the Antarctic skate *Bathyraja eatonii*: new insights into the evolution of globin genes. Gene 406 (1-2), 199-208 IF 2,871
15. Marino S, Hayakawa K, Hatada K, Benfatto M, Rizzello A, Maffia M, Bubacco L (2007) Structural Features that Govern Enzymatic Activity in Carbonic anhydrase from a Low-Temperature Adapted Fish, *Chionodraco hamatus*. Biophys J 93, 2781-2790 IF 4,627
16. Mazzei F, Ghigliotti L, Lecointre G, Ozouff-Costaz C, Coutanceau J-P, Detrich HW III, Pisano E (2007) Karyotypes of basal lineages in notothenioid fishes: the genus *Bovichtus*. Polar Biology 29: 1071-1076 IF 1,734
17. Pavoni E, Cacciarelli D, Tittarelli R, Orsini M, Galtieri A, Giardina B, Brancaccio A (2007) Duplication of the dystroglycan gene in most branches of teleost fish. BMC Mol Biol 8:34 IF 3,371
18. Pisano E, Coscia MR, Mazzei F, Ghigliotti L, Ozouff-Costaz C, Coutanceau J-P, Oreste U (2007) Cytogenetic mapping of Immunoglobulin heavy chain genes in Antarctic fish. Genetica 130 (1):9-17 IF 1,396
19. Rizzello A, Ciardiello MA, Acierno R, Carratore V, Verrio T, di Prisco G, Storelli C, Maffia M (2007) Biochemical

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- characterization of a S-glutathionylated carbonic anhydrase isolated from gills of the Antarctic icefish *Chionodraco hamatus*. *Protein J* 5, 335-348 IF 1,010
- 20. Sanna MT, Manconi B, Poddia G, Olianais A, Pellegrini M, Castagnola M, Messana I, Giardina B (2007) Alkaline Bohr effect of bird hemoglobins: the case of the flamingo. *Biol Chem* 388(8):787-95 IF 2,840
 - 21. Scudiero R, Trinchella F, Riggio M, Parisi E (2007) Structure and expression of genes involved in transport and storage of iron in red-blooded and hemoglobin-less Antarctic Notothenioids. *Gene* 397, 1-11 IF 2,871
 - 22. Verde C, Lecointre G, di Prisco G (2007) The phylogeny of polar fishes and the structure, function and molecular evolution of hemoglobin. *Polar Biol* 30: 523-539 IF 1.734
 - 23. Verde C, Vergara A, Giordano D, Mazzarella L, di Prisco G (2007) The Root effect - A structural and evolutionary perspective. *Antarct Sci* 19: 271-278 IF 1,265
 - 24. Vergara A, Franzese M, Merlino A, Vitagliano L, Verde C, di Prisco G, Lee C-H, Peisach J, Mazzarella L (2007) Structural characterization of ferric hemoglobins from three Antarctic fish species of the suborder Notothenioidei. *Biophys J* 98:2822-2829 IF 4,627
 - 25. De Rosa M. C., Carelli Alinovi C., Galtieri A., Russo A., Giardina B. (2008) Allosteric Properties of Hemoglobin and the Plasma Membrane of the Erythrocyte: New Insights in Gas Transport and Metabolic Modulation. *IUBMB Life*, 60, 87–93
 - 26. Ghigliotti L., Mazzei F., Ozouf-Costaz C., Christiansen J. S., Fevolden S.-E., Pisano E. (2008) First cytogenetic characterization of the sub-arctic marine fish *Mallotus villosus* (Müller, 1776), Osmeriformes, Osmeridae. *Genetics and Molecular Biology* 31, 180-187
 - 27. Giorgi A, Mignogna G, Bellapadrona G, Gattoni M, Chiaraluce R, Consalvi V, Chiancone E, Stefanini S (2008) The unusual co-assembly of H- and M-chains in the ferritin molecule from the Antarctic teleosts *Trematomus bernacchii* and *Trematomus newnesi*. *Arch Biochem Biophys* 478, 69-74
 - 28. Mazzei F., Ghigliotti L., Coutanceau J.-P., Detrich H. W. III, Prirodina V., Ozouf-Costaz C., Pisano E. (2008) Chromosomal characteristics of the temperate notothenioid fish *Eleginops maclovinus* (Cuvier). *Polar Biol* 31, 629–634
 - 29. Merlino A., Verde C., di Prisco G., Mazzarella L., Vergara A. (2008) Reduction of ferric hemoglobin from *Trematomus bernacchii* in a partial bis-histidyl state produces a deoxy coordination even when encapsulated into the crystal phase. *Spectroscopy: Biomed. Appl.* 22, 143-152 (IF 0.597)
 - 30. Trinchella F., Cannetiello M., Simonello P., Filosa S., Scudiero R. (2008) Differential gene expression in *Podarcis sicula* embryos developed under cadmium stress. *Comparative Biochemistry and Physiology (Part A)* 151, S32
 - 31. Trinchella F., Riggio M., Filosa S., Parisi E., Scudiero R. (2008) Molecular aspects of iron acquisition and storage in the cold-adapted Antarctic Notothenioids. *Comparative Biochemistry and Physiology (Part A)* 151, S33
 - 32. Verde C., Giordano D., di Prisco G. (2008) The Adaptation of Polar Fishes to Climatic Changes: Structure, Function and Phylogeny of Haemoglobin. *IUBMB Life* 60, 29–40
 - 33. Verde C., Vergara A., Mazzarella L., di Prisco G. (2008) Hemoglobins of fishes living at polar latitudes: current knowledge on evolutionary and structural adaptation in a changing environment. *Curr. Prot. Pept. Sci.* 9, 578-590 (IF 3.011)
 - 34. Vitagliano L., Vergara A., Bonomi G., Merlino A., Smulevich G., Howes B., di Prisco G., Verde C., Mazzarella L. (2008) Spectroscopic and crystallographic analysis of a tetrameric hemoglobin oxidation pathway reveals features of an intermediate R/T state. *J. Am. Chem. Soc.* 130, 10527-10535 (IF 8.091)
 - 35. Andersen Ø., Wetten O. F., De Rosa M. C., Andre C., Carelli Alinovi C., Colafranceschi M., Brix O., Colosimo A. (2009) Haemoglobin polymorphisms affect the oxygen-binding properties in Atlantic cod populations. *Proc. R. Soc. B* 276, 833-841
 - 36. Bottaro M., Oliveri D., Ghigliotti L., Pisano E., Ferrando S., Vacchi M. (2009) Born among the ice: first morphological observations on two developmental stages of the Antarctic silverfish *Pleuragramma antarcticum*, a key species of the Southern Ocean. *Rev Fish Biol Fisheries* 19, 249–259
 - 37. Cerra M.C., Angelone T., Parisella M.L., Pellegrino D., Tota B. (2009) Nitrite modulates contractility of teleost (*Anguilla anguilla* and *Chionodraco hamatus*, i.e. the Antarctic hemoglobinless icefish) and frog (*Rana esculenta*) hearts. *BBA* 1787, 849–855 (IF 4.447)
 - 38. Cheng C.-H. C., di Prisco G., Verde C. (2009) Cold-adapted Antarctic fish: The discovery of neuroglobin in the dominant suborder Notothenioidei. *Gene* 433, 100–101
 - 39. Cheng C.-H. C., di Prisco G., Verde C. (2009) The “Icefish Paradox.” Which Is the Task of Neuroglobin in Antarctic Hemoglobin-Less Icefish?. *IUBMB Life* 61, 184–188
 - 40. Convey P., Bindschadler R., di Prisco G., Fahrbach E., Gutt J., Hodgson D.A., Mayewski P.A., Summerhayes C.P., Turner J. (2009) The ACCE Consortium “Antarctic climate change and the environment”. *Antarctic Science* 21, 541–563
 - 41. Garofalo F., Amelio D., Cerra M.C., Tota B., Sidell B.D., Pellegrino D. (2009) Morphological and Physiological study of the Cardiac NOS-NO System in the Antarctic (Hb-/Mb-) icefish *Chaenocephalus aceratus* and in the red-blooded *Trematomus bernacchii*. *Nitric Oxide* 20, 69-78 (IF 2.65)
 - 42. Garofalo F., Pellegrino D., Amelio D., Tota B. (2009) The Antarctic hemoglobinless icefish, fifty five years later: A unique cardiocirculatory interplay of disaptation and phenotypic plasticity. *Comp. Biochem. Physiol.* 154 A, 10-28 (IF 1.709)
 - 43. Giordano D., Boechi L., Vergara A., Martí M. A., Samuni U., Dantsker D., Grassi L., Estrin D. A., Friedman J.I M., Mazzarella L., di Prisco G., Verde C. (2009) The hemoglobins of the sub-Antarctic fish *Cottoperca gobio*, a phyletically basal species. Oxygen-binding equilibria, kinetics and molecular dynamics. *FEBS J* 276, 2266–2277

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- 44. Manconi B., Messana I., Maggiani F., Olianas A., Pellegrini M., Crnjar R., Castagnola M., Giardina B., Sanna M. T. (2009) Structural and functional characterization of *Delphinus delphis* hemoglobin system. *J Comp Physiol B* 179, 971–983
 - 45. Merlino A., Vergara A., Sica F., Mazzarella L. (2009) The planarity of heme prosthetic group in proteins: a detailed conformational analysis of Database Protein Structures. *Marine genomics* 2, 51-56
 - 46. Merlino A., Vitagliano L., Howes B., Verde C., di Prisco G., Smulevich G., Sica F., Vergara A. (2009) Combined crystallographic and spectroscopic analysis of *Trematomus bernacchii* hemoglobin highlights analogies and differences in the peculiar oxidation pathway of Antarctic fish hemoglobins. *Biopolymers* 91, 1117-1125 (IF 2.823)
 - 47. Olianas A., Manconi B., Masia D., Sanna M. T., Castagnola M., Salvadori S., Messana I., Giardina B., Pellegrini M. (2009) The oxygen-binding modulation of hemocyanin from the Southern spiny lobster *Palinurus gilchristi*. *J Comp Physiol B* 179, 193–203
 - 48. Quassinti L., Pellegrino D., Garofano F., Maccari E., Bramucci M. (2009) Comparison of angiotensin converting enzyme-like activity in the Antarctic teleosts *Trematomus bernacchii* and *Chionodraco hamatus*. *Polar Biology* 32, 673–677 (IF 1.515)
 - 49. Pisano E., Ghigliotti L. (2009) Ribosomal genes in notothenioid fishes: Focus on the chromosomal organisation. *Marine Genomics* 2, 75–80
 - 50. Scudiero R., Trinchella F., Parisi E. (2009) Iron metabolism genes in Antarctic notothenioids: A review. *Marine Genomics* 1, 79–85
 - 51. Trinchella F., Parisi E., Scudiero R. (2009) Evolutionary analysis of the transferrin gene in Antarctic Notothenioidei: A history of adaptive evolution and functional divergence. *Marine Genomics* 1, 95–101
 - 52. Verde C., Giordano D., Russo R., Riccio A., Vergara A., Mazzarella L., di Prisco G. (2009) Hemoproteins in the cold. *Marine genomics* 2, 67-73
 - 53. Vergara A., Franzese M., Merlino A., Bonomi G., Verde C., Giordano D., Di Prisco G., Lee C., Peisach J., Mazzarella L. (2009) Correlation between Root effect and hemicrome stability in tetrameric hemoglobins. *Biophys. J.* 97, 866-74 (IF 4.683)

B – book chapters

- 1. di Prisco G, Verde C (2006) FISHES Encyclopedia of the Antarctic (Routledge publisher), Taylor and Francis Books, INC., New York
- 2. Pisano E, Ozouf-Costaz C, Foresti F, Kapoor BG (eds) (2007) Fish Cytogenetics, Science Publishers, Inc. Enfield, (NH), U.S.A. (ISBN 978-1-57808-330-5)
- 3. Verde C, di Prisco G (2006) The adaptive evolution of polar fishes: structure, function and molecular phylogeny of hemoglobin. Memoirs of National Institute of Polar Research, Special Issue No. 59, 16-28
- 4. di Prisco G, Verde C (2007) Predicting the impacts of climate change on the evolutionary adaptations of polar fish. In Life in Extreme Environments (Eds. Amils R; Ellis-Evans C; Hinghofer-Szalkay, HG) Reprinted from Reviews in Environmental Science and Bio/Technology. Springer
- 5. Verde C, Giordano D, di Prisco G (2007) Molecular evolution of haemoglobins of polar fishes. In Life in Extreme Environments (Eds. Amils R; Ellis-Evans C; Hinghofer-Szalkay, HG) Reprinted from Reviews in Environmental Science and Bio/Technology. Springer
- 6. Dettaì A., di Prisco G., Lecointre G., Parisi E., Verde C. (2008) Inferring Evolution of Fish Proteins: The Globin Case Study. Edited by R.K. Poole "Spectroscopic and crystallographic characterization of hemicromes in tetrameric hemoglobins". *Meth. Enz.* (Part A) 436, 539-570 (IF 2.126)
- 7. Negrisolo E., Bargelloni L., Patarnello T., Ozouf-Costaz C., Pisano E., di Prisco G., Verde C. (2008) Comparative and Evolutionary Genomics of Globin Genes in Fish. Edited by R.K. Poole "Spectroscopic and crystallographic characterization of hemicromes in tetrameric hemoglobins". *Meth. Enz.* (Part A) 436, 511-538 (IF 2.126)
- 8. Vergara A., di Prisco G., Verde C., Mazzarella L. (2008) Bis-Histidyl adducts in tetrameric hemoglobins. In Series *Protein Reviews*, Springer (Series Editor: M. Zouhair Atassi) "A Tribute to Beatrice and Jonathan Wittenberg", Eds M. Bolognesi, G. di Prisco, C. Verde, vol. 2, Cap. 10, 93-106
- 9. Vergara A., Vitagliano L., di Prisco G., Verde C., Mazzarella L. (2008) Globins and other nitric oxide-reactive proteins. Edited by R.K. Poole "Spectroscopic and crystallographic characterization of hemicromes in tetrameric hemoglobins". *Meth. Enz.* (Part A) 436, 421-440 (IF 2.126)

C - proceedings of international conferences

- 1. Marino K, Cocca E (2006) Organisation of the Hb1 genes in the Antarctic skate *Bathyraja eatonii* : a new contribution towards the understanding of the evolution of the globin gene family. *Marine Genomics – An International Conference*, Sorrento, Italy. Poster 79, pag. 105-106
- 2. Scudiero R, Trinchella F, Riggio M, Parisi E (2006) Structure and Expression of Genes Involved in Transport and Storage of Iron in Red-blooded and Haemoglobinless Antarctic Notothenioids. *Marine Genomics – An International Conference*, Sorrento, Italy.

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3. Vergara A, Merlino A, Franzese M, di Prisco G, Verde C, Peisach J, Lee C, Mazzarella L (2006) Stereochemistry, formation and reactivity of hemichromes in Antarctic fish hemoglobins. XIVth International Conference on Dioxygen binding and sensing proteins, Napoli, Italy
4. di Prisco G, Danis B, De Broyer C, Dettai A, Ellis-Evans C, Huiskes A, Verde C, Wilmette A (2007) FP7: Research on climate change in polar environments must include effects on biota of both polar regions. International Symposium Polar Environment and Climate: The Challenges European Research in the Context of the International Polar Year. Brussels, Belgium, pp 140-142
5. di Prisco G, Verde C (2007) EBA: Evolution and Biodiversity in the Antarctic
6. di Prisco G, Verde C (2007) The response of life to change - An overarching SCAR-IPY Programme. International Symposium Polar Environment and Climate: The Challenges European Research in the Context of the International Polar Year. Brussels, Belgium, pp 137-139
7. Ghigliotti L, Mazzei F, Ozouf-Costaz C, Cheng C-H, Christiansen JS, Fevolden S-E, Babiak I, Pisano E (2007) Insights into the chromosomal features of Atlantic cod. XII European Congress of Ichthyology, Dubrovnik, Abstracts: 56
8. Mazzei F, Ghigliotti L, Ozouf-Costaz C, Coutanceau J-P, Detrich HW III, Prirodina VP, Pisano E (2007) Chromosomal characteristics of *Eleginops maclovinus*, a basal, sub-Antarctic notothenioid. PNRA Antarctic Biology Meeting, Follonica, Italy
9. Pisano E (2007) From cytogenetics to cytogenomics of Antarctic fishes. PNRA Antarctic Biology Meeting, Follonica, Italy
10. Verde C, di Prisco G (2007) Coping with cold. The evolution of hemoglobin in fishes living at polar latitudes. International Symposium Polar Environment and Climate: The Challenges European Research in the Context of the International Polar Year. Brussels, Belgium, pp 151-153
11. Vergara A, Merlino A, Franzese M, di Prisco G, Verde C, Peisach J, Lee C, Mazzarella L (2007) Linkage between Root effect and hemichrome stability. VII European Symposium of the Protein Society, Stoccolma, Sweden
12. Vergara A, Merlino A, Vitagliano L, Zagari A, Mazzarella L (2007) A combined Raman microscopy and X-ray crystallography approach to study protein single crystals. XIIth European Conference on the Spectroscopy of Biological Molecules, Paris, France
13. Bottaro M., Vannini G., Pisano E., Bono R., Vacchi M. (2008) The coastal fish assemblages at terra Nova Bay in sea-ice and open water conditions XXX SCAR Open Science Conference, St. Petersburg, Abstracts: 302.
14. Ghigliotti L., Cheng C.-H.C., Christiansen J.S., Fevolden S.-E., Mazzei F., Pisano E. (2008) First cytogenetic information on Arctic fishes of the genus *Lycodes* (Zoarcidae). XXX SCAR Open Science Conference, St. Petersburg, Abstracts: 359
15. Ozouf-Costaz C., Hautecoeur M., Coutanceau J.P., Bonillo C., Ghigliotti L., Dettai A., Duhamel G., Pisano E. (2008) Evolution of karyotypic changes in the Paranotothenia/Notothenia clade. XXX SCAR Open Science Conference, St. Petersburg, Abstracts: 347
16. Pisano E (2008) Genomic evolution in Antarctic fish: a cytogenomic perspective. International EBA workshop "The polar and alpine environment: molecular and evolutionary adaptations in prokaryotic and eukaryotic organisms", May, Naples, Italy
17. Pisano E., Ghigliotti L., Bono R., Spirandelli E., Bottaro M., Vacchi M. (2008) Fish Life Under The Antarctic Ice. XXX SCAR Open Science Conference, St. Petersburg, Abstracts: 464

D – proceedings of national meetings and conferences

1. Maffia M, Rizzello A, Acierno R, Verri T, Storelli C (2006) Intestinal peptide transporter of the antarctic haemoglobinless teleost *Chionodraco hamatus*. 55° Congresso Nazionale della Società Italiana di Fisiologia, Pisa, Italia. Acta Physiologica (Oxford). 188 (Suppl. 652), p. 191
2. Maffia M, Rizzello A, Verri T, Storelli C, Acierno R (2006) Molecular aspects of the adaptation to low temperature of functional protein in Antarctic poikilotherms. 57° Congresso Nazionale della Società Italiana di Fisiologia. Ravenna, Italia. Acta Physiologica (Oxford). 188 (Suppl. 652), p. 83
3. Marino S, Hayakawa K, Hatada K, Benfatto M, Rizzello A, Maffia M, Bubacco L (2006) Structural features that govern enzymatic activity of Carbonic anhydrase in a low temperature adapted fish *Chionodraco hamatus*. Acta Biophysica Romana Roma, Università degli Studi di Roma "Tor Vergata", Roma, Italia
4. Rizzello A, Ciardiello MA, Acierno R, Carratore V, Verri T, di Prisco G, Storelli C, Maffia M (2006) Structural and functional characterisation of gill carbonic anhydrase of the Antarctic icefish *Chionodraco hamatus*. 57° Congresso Nazionale della Società Italiana di Fisiologia. Ravenna, Italia. Acta Physiologica (Oxford). 188 (Suppl. 652), p. 12
5. Scudiero R, Trinchella F, Riggio M, Parisi E (2006) Studio del metabolismo del ferro nei teleostei antartici privi di emoglobina: differenze e somiglianze con i teleostei a sangue rosso. 67° Congresso Nazionale UZI, Napoli, Italia
6. Vergara A, Merlino A, Franzese M, di Prisco G, Verde C, Peisach J, Lee C, Mazzarella L (2006) A combined EPR and X-ray crystallographic approach to the reactivity of hemichromes in Antarctic fish hemoglobins. XXII Congresso Nazionale della Società Chimica Italiana, Firenze, Italia
7. Ghigliotti L, Mazzei F, Pisano E (2007) Caratterizzazione citogenetica di *Gadus morhua* L., 1758 (Gadiformes, Gadidae). Atti 68° Convegno Nazionale dell'Unione Zoologica Italiana, Lecce, Italia
8. Pisano E, Ghigliotti L, Mazzei F (2007) Evoluzione genomica nei pesci: il contributo della "nuova citogenetica".

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- Atti 68° Convegno Nazionale dell'Unione Zoologica Italiana, Lecce, Italia
- 9. Rizzello A, Romano A, Kottra G, Acierno R, Ferrara A, Verri T, Storelli C, Daniel H, Maffia M (2007) Molecular and functional characterization of an intestinal peptide transporter in the Antarctic icefish (*Chionodraco hamatus*). 58° Congresso Nazionale della Società Italiana di Fisiologia. Lecce, Italia. *Acta Physiologica (Oxford)*. 191 (Suppl. 657), pp 31-32
 - 10. Pisano E., Ghigliotti L., Mazzei F. (2008) Riorganizzazione cromosomica dei geni ribosomal durante l'evoluzione dei teleostei Notothenioidei. 69° Congresso UZI, Senigallia, Abstracts: 54-55
 - 11. Ghigliotti L., Near T.J., Vacchi M., Pisano E. (2009) Lights on the karyotypic evolution within the teleostean family Artedidraconidae. 70° Congresso UZI, Rapallo (Genova), Abstracts: 140

E – thematic maps

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F – patents, prototypes and data bases

Nucleotide sequences deposited in Gene Data Bank (GeneBank)

- 1. DQ481240 - *Bathyraja eatonii* alpha-globin gene, exons II, III and partial cds; and beta-globin gene, exons I, II and partial cds (2006)

Crystal structures deposited in Protein Data Bank (PDB)

- 1. 2H8F. Crystal structure of the deoxy hemoglobin isolated from the Antarctic fish *Trematomus bernacchii* at pH=6.0 (2006)
- 2. 2H8D. Crystal structure of the deoxy hemoglobin isolated from the Antarctic fish *Trematomus bernacchii* at pH=8.4 (2006)
- 3. 2PEG. High resolution crystal structure of a tetrameric hemoglobin from *Trematomus bernacchii* in a hemichrome state (2007)
- 4. 3D1K. Nearly atomic resolution structure of a partially liganded hemoglobin from *Trematomus newnesi* in a R-T intermediate state (2008)
- 5. 3GKV. X-ray structure of an intermediate along the oxidation pathway of *Trematomus bernacchii* hemoglobin (2009)
- 6. 3GQG. Crystal structure at acidic pH of the ferric form of the Root effect hemoglobin from *Trematomus bernacchii* (2009)

G – exhibits, organization of conferences, editing and similar

Organization of conferences

- 1. "XIV International Conference on Dioxygen Binding and Sensing Proteins" September 2006, Naples, Italy

Editing

- 1. Dioxygen Binding and Sensing Proteins, Protein Reviews Series (2007) (Eds. Bolognesi M, di Prisco G, Verde C) Springer
- 2. Gene, Special Issue devoted to the XIVth International Conference on Dioxygen Binding and Sensing Proteins (2007) (Eds. Moens L, Bolognesi M, di Prisco G, Verde C) Elsevier
- 3. di Prisco G, Luporini P, Tutino L, Verde C. (2009) Editor's Comment - Marine Genomics 2, iii
- 4. di Prisco G, Verde C. (2009) Biodiversity's role in a sustainable world. Intern Innov, 49-51

Speeches

- 1. C Verde: Structure, Function and Molecular Evolution of the Hemoglobins of Polar Fishes (2006) Invited Speaker. XIV International Conference on Dioxygen Binding and Sensing Proteins, Napoli, Italy
- 2. C Verde: ICEFISH 2007 (2006) Invited Speaker. Workshop on Antarctic Evolutionary Biology, Leuven, Belgium
- 3. C Verde: Structure, function and molecular phylogeny of haemoglobin in sub-Antarctic and temperate notothenioid fish (2006) Speaker. Marine Genomics, Sorrento, Italy
- 4. C Verde: Tracking Adaptive Evolution in the Structure, Function and Molecular Phylogeny of Hemoglobin in non-Antarctic Notothenioid Fish Species (2006) Invited Speaker. XXIX SCAR Meeting and Open Science Conference, Hobart, Australia
- 5. C Verde: International Collaborative Expedition to collect and study Fish Indigenous to Sub-Antarctic Habitats: ICEFISH-2007 (2006) Invited Speaker. National Conference on research in polar areas, Rome, Italy
- 6. A Vergara: Stereochemistry, formation and reactivity of hemichromes in Antarctic fish hemoglobins (2006) Keynote Speaker. XIVth International Conference on Dioxygen binding and sensing proteins, , Naples, Italy
- 7. C Verde: International Collaborative Expedition to collect and study Fish Indigenous to Sub-Antarctic Habitats (ICEFISH) (2007) Invited Speaker. 6th PNRA Meeting on Antarctic Biology, Follonica, Italy

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8. C Verde: Life and adaptations to polar environments (2007) Keynote Speaker. Italy-Norway Meeting, Rome, Italy
9. C Verde: Climate change and the polar ichthyofauna: molecular evolution of hemoglobin (2007) Keynote Speaker. Ny-Ålesund and IPY" Seminar, Cambridge, UK

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

PhD thesis

1. G Barbiero: Structure and function of hemoproteins in polar organisms (2007) Dept. of Chemistry, Univ. "Federico II", Naples, Italy. Tutors: Prof L Mazzarella e Dr C Verde
2. K Marino: Expression, isolation and characterization of tetrameric haemoglobin as a model to study the Root effect (2007). Dept. of Chemistry, Univ. "Federico II", Naples, Italy. Tutors: Prof L Mazzarella e Dr E Cocca

Post-doc fellowship

1. D Giordano: Structure and function of hemoproteins in polar organisms (2007-9) Institute of Protein Biochemistry - CNR, Naples, Italy
2. G Bellapadrona: Antarctic fish ferritin: adaptation mechanisms to the temperature (2008) Institute of Molecular Biology and Pathology – CNR, Rome, Italy
3. P Ceci: Bacterial ferritin: adaptation mechanisms to the temperature (2009) Institute of Molecular Biology and Pathology – CNR, Rome, Italy
4. L Ghigliotti: Cytogenetic and molecular study in polar teleost (2009) University of Genoa, Italy

Research fellowships

1. D Coppola: Structure and function of hemoproteins in polar organisms (2007-9) Institute of Protein Biochemistry - CNR, Naples, Italy
2. F Mazzei: Cytogenetic study of Antarctic and Subantarctic teleosts from the campaign ICEFISH 2004 (2006) University of Genoa, Italy
3. L Ghigliotti: Cytogenetic and molecular study of 5S ribosomal genes in polar teleost (2006) University of Genoa, Italy
4. A Riccio: Structural and functional characterization of hemoglobins isolated from Arctic fish (2007-9) Institute of Protein Biochemistry - CNR, Naples, Italy
5. F Trinchella: Study the expression of genes involved in iron metabolism in Antarctic Osteichthyes without hemoglobin (2009) University Federico II, Dept. of Biological Sciences, Naples, Italy

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Date: April 26, 2010

Notes

The products of research activity were not completely updated.