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Glycoprotein antifreeze in *Notothenia coriiceps* (Pisces: Nototheniidae) from the sub-Antarctic Marion Island

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Research on Notothenia coriiceps *from the sub-Antarctic Marion Island suggests that its blood contains antifreeze glycoprotein (AFGPs) and its kidneys are aglomerular. This is the first time both features of freezing avoidance have been found in fish distributed north of the Antarctic Polar Front. The results of this study favour the argument that Antarctic and sub-Antarctic stocks of* Notothenia coriiceps *represent a single species.*

Navorsing op Notothenia coriiceps *vanaf die sub-Antarktiese Marion Eiland stel voor dat hul bloed 'n vrieswerende middel, genoem glikogeen-proteïen (AFGPs) bevat en dat hul niere aglomerular is. Dit is die eerste keer dat beide verskynsels van bevriesingsvermyding in visse wat noord van die Antarktiese Poolstreke voorkom, gevind is. Die bevindinge van hierdie studie is ten gunste van die argument dat Antarktiese en sub-antarktiese subbevolkings van* Notothenia coriiceps *'n enkele spesie verteenwoordig.*

Introduction

Antarctic fishes, living in the icy water around the Antarctic continent, avoid freezing by producing proteins that lower the freezing point of their body fluids below that of seawater (DeVries 1971). These proteins are synthesised in the liver (O'Grady *et al* 1982) and are distributed in all interstitial fluids of the body excluding the nervous system (Ahlgren *et al* 1988). Two major types of biological antifreeze agents are produced by Antarctic fishes. Notothenioid fishes produce antifreeze glycoproteins (AFGPs), whereas other groups, including gadiform, myctophiform and scorpaeniform species, use proteins lacking the sugar component (AFPs) (DeVries & Lin 1977; Wöhrmann 1993).

To date 54 species of fishes, collected in Antarctic continental waters, Chile, Australia and New Zealand, have been investigated for the presence of antifreeze proteins (Eastman 1993; Wöhrmann 1993). Of these, 49 species, living in water of 2 °C or colder and in which ice may be encountered during the year, produce AFGPs and AFPs.

The suborder Notothenioidei, comprising 34% of all Southern Ocean species, is the largest single group of Antarctic fishes, the majority of which (about 80%) are endemic to the Antarctic region (Gon & Heemstra 1990). Forty-seven species, representing 38% of the species in this suborder, have been investigated for the presence of AFGPs (Eastman 1993; Wöhrmann 1993). AFGPs were found in 42 species distributed south of the Antarctic Polar Front. The five remaining species, with cold temperate distribution (i e Australia, New Zealand and Chile), lack AFGPs (Eastmann 1993; Wöhrmann 1993).

The state of glomerular development was examined in 25 of the same 47 species (Eastman 1993). Twenty species, found south of the Antarctic Polar Front, had aglomerular kidneys. Five species had glomerular kidneys and occur only north of the Antarctic Polar Front. The same pattern was found in eight additional notothenioid species (not checked for the presence of AFGPs).

The geographical distribution of notothenioid species with AFGPs and aglomerularism implies that both features are present only in Antarctic species, i e species likely to encounter ice some time during their life (Eastman 1993). The present paper implies that *Notothenia coriiceps* from the ice-free water of the sub-Antarctic Marion Island in the Indian sector of the Southern Ocean has AFGPs and aglomerular kidneys. It also demonstrates that antifreeze research can play a role in resolving taxonomic and biogeographical questions in Antarctic ichthyology.

Materials and methods

Specimens of *N. coriiceps* were collected by hand while scuba-diving at Ship's Cove, Marion Island (46°45'S, 37°55'E) in 1989 (Gon & Mostert 1992).

Blood was collected by heart puncture from three specimens. The samples were pooled and allowed to clot for two hours at 0 °C. The clot was spun down and the serum, divided into two subsamples of about 5,0 ml each, was stored in -20 °C. After thawing, residual serum proteins were removed by treating each subsample with 10% trichloroacetic acid (TCA) followed by centrifugation at 220 000 x g for two hours. The TCA was removed by dialysing three times against a 2,5 mM Tris-HCl pH 9,2 starting buffer. Each subsample was then passed over Whatman DEAE 52 ion exchange resin (column dimensions: 1,5 x 75 cm) equilibrated with 2,5 mM Tris-HC1 pH 9,2 buffer. The column was rewashed with a 50 mM Tris-HCl pH 9,2 buffer to elute remaining antifreeze molecules. Fractions, each 2,0 ml in volume, were collected after an initial non UV-absorbing volume of 20 ml from the column was discarded. A Unicam SP 800 Spectrophoto-meter was used to measure the absorbence of the collected fractions at 230 nm and 280 nm.

Kidneys were removed from two specimens, fixed in 10% formalin and embedded in paraffin wax. Cutting was done serially in sections of 5 and 10 µm. The sections were stained in haematoxylin and phloxine, and examined under a light microscope for the presence of glomeruli. The slides of the kidney sections are deposited in the collection of the J L B Smith Institute of Ichthyology, Grahamstown (RUSI 18233, 18234).

Results

The ion exchange chromatography of both serum subsamples showed similar results. Elution with the starting buffer (2,5 mM Tris-HCl pH 9,2) showed a single peak at 280 nm in fraction 3; at 230 nm two major (fractions 3 and 18-31) and two minor (fractions 8, 12) peaks were resolved (Fig 1). Two minor peaks (fractions 34-35, and 45-48) eluted with the concentrated buffer (50 mM Tris-HCl pH 9,2).

This elution profile (Fig 1) is comparable to the profile Ahlgren & DeVries (1984: Fig 1) obtained from serum of *N. coriiceps* from the Antarctic Peninsula. Although absorbence values at 230 nm in the author's profile are lower (due to the long column used), peak height ratio of the two major peaks agrees with that of Ahlgren & DeVries 1984. In both studies the first peak eluted immediately after the void volume. The strength of buffer required to elute the low molecular weight AFGPs varies (A L DeVries pers comm), hence the difference between the two studies regarding the position of the second peak relative to the change in buffer concentration. The similarity between the elution profiles of both studies suggests that *N. corriiceps* from Marion Island has a similar distribution of the same AFGPs found in Antarctic Peninsula fish. Consequently, in the authors' profile fraction 3 (first major peak) represents the high molecular weight AFGPs 1-5 and fractions 18-31 (second major peak) represent the low molecular weight AFGPs 6-8.

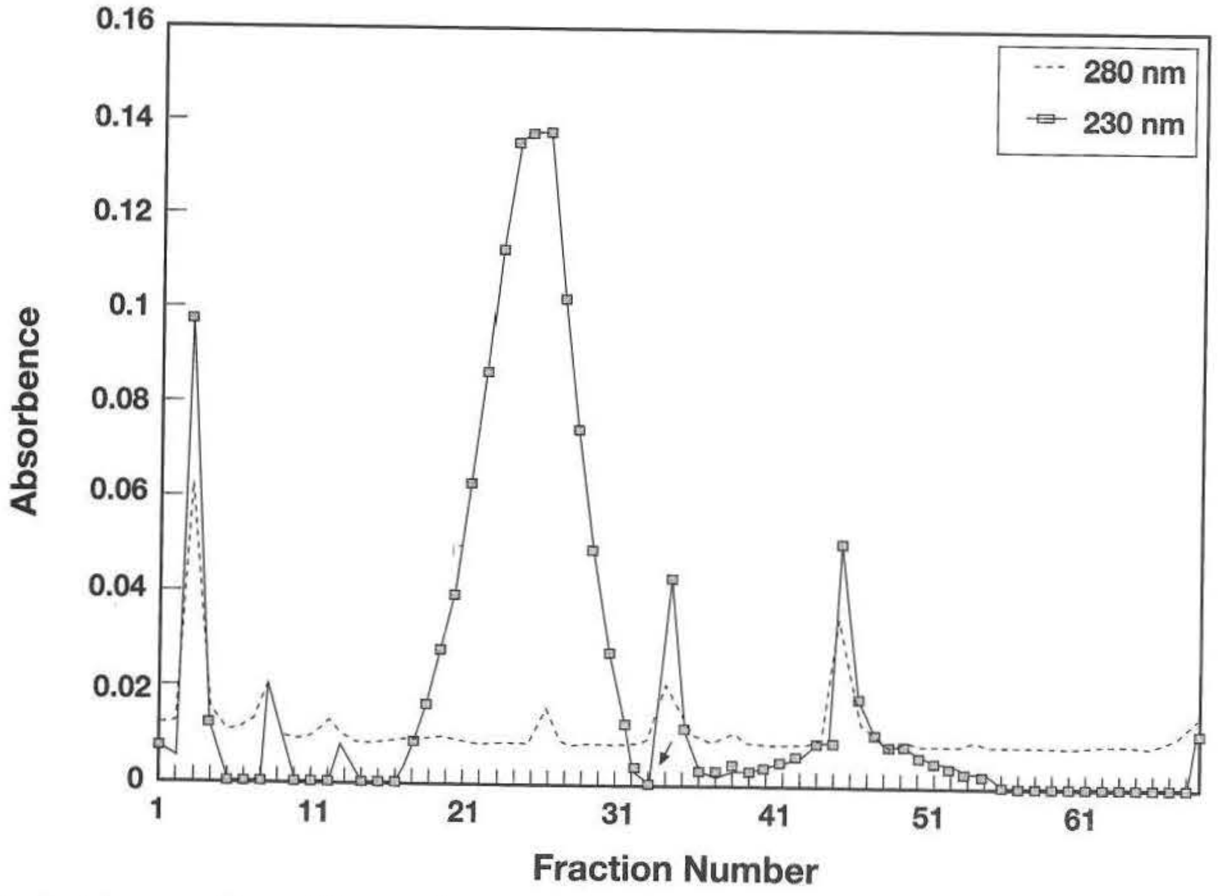

Fig 1. Absorbence profiles at 230 nm and 280 nm of semi-purified serum from Nothothenia coriiceps *after elution from a Whatman DEAE 52 ion exchange column; the initial concentration of the buffer, 2,5 mM Tris-HCl pH 9,2, was raised to 50 mM at fraction 33 (arrow)*

Examination of the kidney sections revealed that both kidneys are aglomerular (Fig 2), thus providing further evidence for the presence of AFGPs in these fishes.

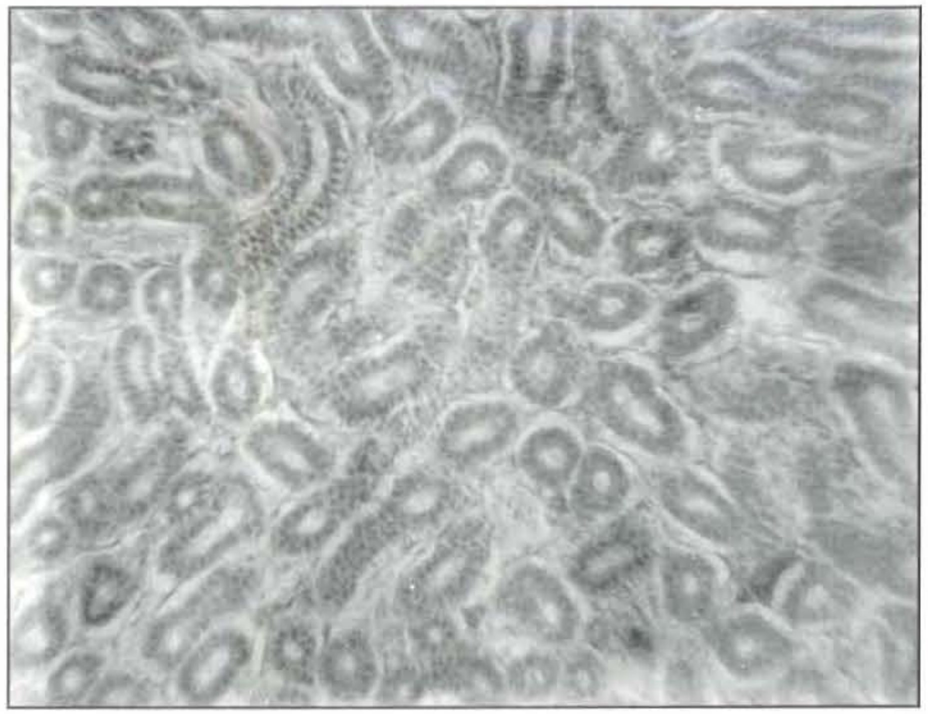

Fig 2. Aglomerular kidney of Notothenia coriiceps *from Marion Island; haematoxylin and phloxine*

Discussion

The aglomerular kidney, though unusual, is not unique to Antarctic fishes. This type of kidney is known to occur in a small number of unrelated freshwater and marine fish species (Hickman & Trump 1969). AFGPs, however, are unique to polar fishes. Although there is no direct proof that the presence of AFGPs and aglomerular kidneys in notothenioid fishes coevolved, current knowledge favours such an hypothesis (Eastman 1993).

Eastman (1993) examined the apparent relationship between the presence of AFGPs and aglomerular kidneys in notothenioid fishes on two levels. His statistical analysis suggests a non-random association between these two attributes. His phyletic analysis shows that the distribution of AFGPs and aglomerularism within the Notothenioidei is correlated with geographical distribution and phylogenetic position. Generally, taxa distributed north of the Antarctic Polar Front are phyletically primitive and lack both AFGPs and aglomerular kidneys. Derived taxa, found in Antarctic waters, have both. An interesting exception to these conclusions was found in the genus *Patagonotothen*, currently regarded as the most primitive genus in the subfamily Nototheniinae (Balushkin 1984), which has a cold temperate to sub-Antarctic distribution (i e the southern part of South America, South Georgia and Shag Rocks). *P. cornucola*, distributed in southern South America and the only species investigated so far in this genus (Eastman 1993), has aglomerular kidneys (not tested for AFGPs).

Past antifreeze research, on which Eastman's (1993) analyses were based, had two limitations. Firstly, it concentrated on species living at the southern and northern extremes of notothenioid geographical range (e g Antarctic continental shelf, New Zealand, Chile). Little attention was given to species inhabiting sub-Antarctic and cold temperate waters (e g South Georgia, islands of the Indian Ocean sector, southern tip of South America). Secondly, the majority of species studied to date occur either south or north of the Antarctic Polar Front. Studies involving wide-ranging species distributed in both regions are few and incomplete (i e samples were only collected in a single locality; either AFGPs or aglomerularism were investigated, but not both).

Nybelin's (1951) splitting of Antarctic and sub-Antarctic stocks into *Notothenia neglecta* and *N. coriiceps*, respectively, created a long-lasting controversy regarding the taxonomic status of these nominal sister species. Considering the case of *Dissostichus mawsoni* (Antarctic) and *D. eleginoides* (sub-Antarctic to temperate), two sisters species that fit Eastman's (1993) model of latitudinal segregation in freezing avoidance, AFGPs and aglomerularism would be expected to occur in *N. neglecta*, but not in *N. coriiceps* (sensu Nybelin), which inhabits the ice-free water of Marion Island. Their presence in fishes of both Nybelin's taxa suggests one widespread species, i e *Notothenia corriiceps* (Richardson 1844), as has been argued by Gon & Klages (1988) and De Witt *et al* (1990).

The presence of AFGPs and aglomerular kidneys in *N. corriiceps* from Marion Island is not really surprising and could have been anticipated. The geographical position of Marion Island makes it the first sub-Antarctic land mass encountered by organisms carried by the Antarctic Circumpolar Current (West Wind Drift) from South America. The notohenioid component of the island's fish fauna originated from the Patagonia-Falkland Islands region and the Scotia Sea and was carried across by this current (Gon & Klages 1988). Marion Island is geologically young, 0,25-0,50 Ma (McDougal 1971) and the sea around it is ice-free throughout the year (Schulze 1971; Anon 1992). Given that (1) colder conditions during the glacial periods of the Pleistocene may have been more conducive to a north-west migration of Scotia Arc and Patagonian notothenioids, and enhanced extralimital survival, and that (2) those migrants, including *N. coriiceps*, evidently endure water temperature as much as 5 °C above that of their original area, a time span of 0,5 Ma is probably not long enough for freezing avoidance to have been lost even though it is not needed in sub-Antarctic water.

An alternative hypothesis is that the Antarctic Polar Front does not comprise a thermal barrier for *N. coriiceps* and that having AFGPs and aglomerular kidney is not overtly detrimental to the population of Marion Island. Evidence that *N. coriiceps* and *N.rossii* from Kerguelen Islands also have aglomerular kidney (Gon, unpublished data) supports this hypothesis.

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Preliminary biological survey of Vesleskarvet, northern Ahlmannryggen, western Queen Maud Land: site of South Africa's new Antarctic base

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The results of a preliminary biological survey of Vesleskarvet, a nunatak in the northern Ahlmannryggen mountain range of Queen Maud Land, are presented. Vesleskarvet is the site of the new SANAE IV base and the biological survey preceded base construction.

Soil nutrient levels are low and plant growth is very sparse, with only nine species of lichen and one, as yet unidentified, species of moss being recorded to date. Bacteria, microalgae, fungi, rotifers and ciliates were counted from soil samples. No collembolans and only one specimen of mite, suspected to be Tydeus sp.*, were found at Vesleskarvet. However, three species of Tardigrada, including two previously undescribed species, and three nematode species, one of which represents a new genus, were recorded at the site. Four species of bird were seen in the vicinity of Vesleskarvet but none breeds at the nunatak and only one, the snow petrel* Pagodroma nivea*, is commonly seen.*

Although Vesleskarvet supports only a limited biota, which fact contributed to its selection as the site for a base, it is an example of an uncommon and very little studied ecosystem. Therefore, it is advised that the site be declared a Specially Managed Area in terms of the Protocol on Environmental Protection to the Antarctic Treaty of 1991.

Introduction

The present South African National Antarctic Research Base, SANAE III (70°18'S, 02°21'W) in Queen Maud Land, which became operational in early 1979, is now due for replacement (Claassen & Sharp 1993). To avoid the problems of snow accumulation and the sinking of buildings built on ice shelves, with the subsequent stresses exerted on structures (Claassen & Sharp 1993), a new base, SANAE IV, is presently being constructed on exposed rock at an inland nunatak. The Vesleskarvet nunatak (71°40'S, 02°51'W), some 160 km inland of SANAE III (Figure 1), was selected as the site for the new base and therefore, during the 1991/92 and 1992/93 summers, an environmental impact assessment was conducted at this nunatak in accordance with the Protocol on Environmental Protection to the Antarctic Treaty of 1991. The biological component of the assessment forms the basis of this paper.

Terrestrial biological research by South Africa in Queen Maud Land has only recently been initiated, with the South African Biological Antarctic Research Subprogramme (SABARSP) commencing in the 1991/92 summer field season (Cooper *et al* 1991). Prior to this the only detailed terrestrial biological research conducted by South Africa in Antarctica was a biological survey of Robertskollen (71°28'S, 03°15'W) (Ryan *et al* 1989), a group of nunataks in the north-western Ahlmannryggen approximately 25 km to the north-northwest of Vesleskarvet (Fig 1).

Study area

Vesleskarvet was apparently named by the 1949-52 Norwegian-British-Swedish Antarctic Expedition, although the nunatak was not actually visited at that time. Alberts (1981) gave the meaning of the name as "little barren mountain" but a literal translation from Norwegian is a small, or little, cliff (*vesle* = small and *skarvet* = cliff, S-H Lorentsen pers comm). As far as can be ascertained, Vesleskarvet was first visited by scientists in 1971/72 (Schaefer 1973), and has since been visited several times by South African earth sciences field teams (Schonfeld & Van Zyl 1974, J R Krynauw pers comm).

The Vesleskarvet group of nunataks is situated in the northern Ahlmannryggen of west-central Queen Maud