Antarctic Science 20 (5), 437–440 (2008) © Antarctic Science Ltd 2008 Printed in the UK doi:10.1017/S0954102008001016

Circumpolar occurrence of eugregarinid protozoan *Cephaloidophora pacifica* associated with Antarctic krill, *Euphausia superba*

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Abstract: The geographical distribution of protozoan parasite *Cephaloidophora pacifica* Avdeev (Order Eugregarininda) associated with Antarctic krill, *Euphausia superba*, was examined in samples collected from the vicinity of the Antarctic Peninsula, near Syowa Station, and Pacific and Indian sectors of the Southern Ocean. *Cephaloidophora pacifica* was found at all stations around the Antarctic, with 96.4% of the euphausiids infected ($n = 195$). The numbers of *C. pacifica* per krill ranged from 0 to 8089 krill^{-1}, and the average was 350.0 ± 787.8 (mean $\pm$ SD). The frequency distributions of *C. pacifica* showed an overdispersed parasite population (i.e. the variance was greater than the mean) at all locations. Statistical analysis showed that whilst the geographical location did not have a significant effect on intensity of *C. pacifica* the maturity stage of krill did, with an increasing intensity of infection as krill matures. The infestation of *E. superba* by eugregarinid protozoan is considered to be a circum-Antarctic phenomenon, and it occurs equally throughout the Southern Ocean.

Received 4 April 2007, accepted 30 August 2007

Key words: *Cephaloidophora indica*, geographical distribution, maturity stage, parasite, Southern Ocean

Introduction

Gregarines (Phylum Apicomplexa, Class Sporozoea) are protozoans found in the digestive tracts and body cavities of various planktonic invertebrates including Polychaeta, Chaetognatha, Crustacea, Mollusca, and Tunicata (see Théodoridès 1989). The sub-class Gregarinia is divided into four orders, Blastogregarinida, Archgregarinida, Eugregarinida and Neogregarinida on the basis of life cycle characteristics and cytomorphological differences (Hausmann & Hülsmann 1996). The life cycles of eugregarines associated with terrestrial insects are well known. For example, *Gregarina korogi* Hoshide, an eugregarinid parasite of the cricket, *Gryllus yemma* Ohmachi et Matsuura, passes through five stages: sporozoite, cephaline, gamont, gametocyst and oocyst (Hoshide & Todd 1993). The first three stages occur inside the host's digestive tract and the latter two stages outside the host. The oocyst is ingested by the host and enters the digestive tract. The sporozoites emerge from the oocyst and attach to epithelium cells. This stage is referred to as the cephaline. After detachment from epithelium cells, the cephaline grows into a motile stage, the gamont. Gamonts join together in pairs (syzygy stage) and form a gametocyst, which loses its motility. At the gametocyst stage, eugregarines are excreted with the host's faeces. Then the gametocyst discharges many oocysts.

Avdeev (1985) found two eugregarinid species, *Cephaloidophora pacifica* Avdeev and *Cephaloidophora indica* Avdeev, in the digestive tract and mid-gut gland of Antarctic krill, *Euphausia superba* Dana. The first species was found in the area of Elephant Island (55ºE; Atlantic sector) and westwards to Balleny Islands (175ºW; Pacific sector), and the western part of the Indian sector (30º–68ºE). The second species was found only in waters of the eastern part of the Indian sector (110º–148ºE). Dolzhenkov *et al.* (1987) pointed out that the geographical differences in the occurrence of the two eugregarinids might be used as a biological indicator of the structure of krill population.

The intensity of *C. pacifica* has been known to increase significantly with the maturity of the host (Takahashi *et al.* 2004). Avdeev & Vagin (1987) found a significant correlation in numbers between eugregarines and clots in diverticula, and suggested the eugregarines had a pathological effect on krill. Electron microscopic observations by Kawaguchi (unpublished observations) revealed that *C. pacifica* in the mid-gut gland may cause destruction of microvilli on the surface of hepatic cells, and those in the hind-gut may absorb nutrients from the epithelial cells. *Euphausia superba*

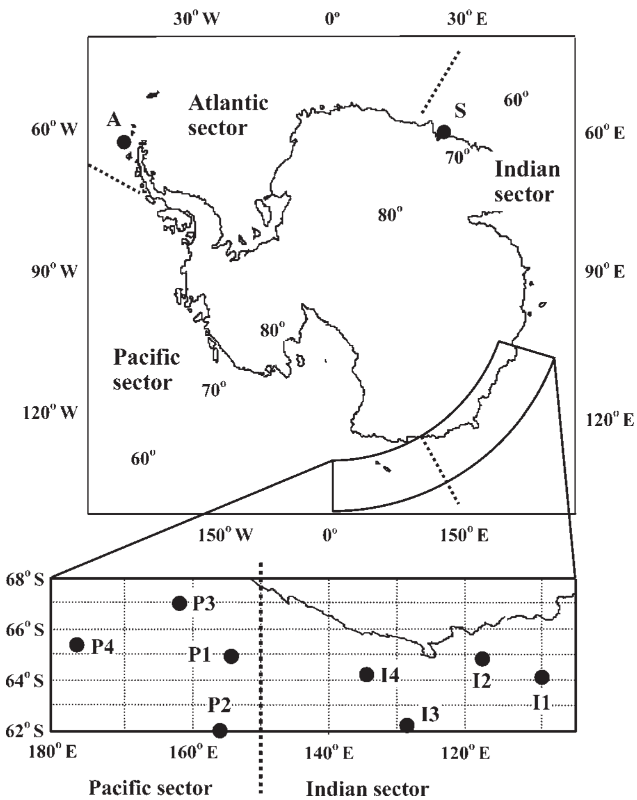

Fig. 1. Locations of the samples used in the present study, A = Antarctic Peninsula, S = Syowa Station, I1-I4 = Indian sector, P1-P4 = Pacific sector.

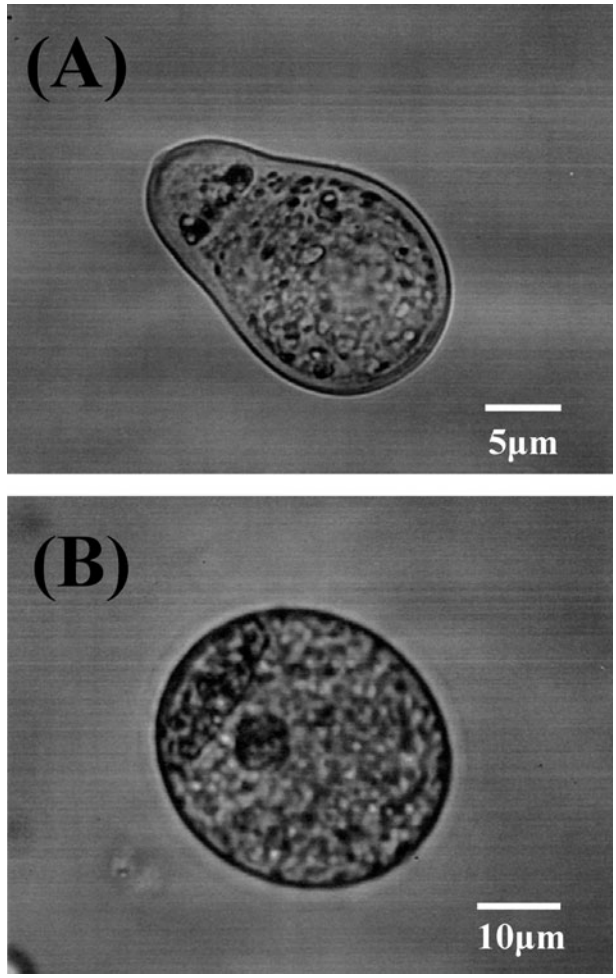

Fig. 2. a. Immature gamont, and **b.** mature gamont of *Cephaloidophora pacifica*.

is considered to be a key species of the Antarctic ecosystem because of its high biomass and important role (Hempel 1985). The occurrence of eugregarines and their negative impact on krill are therefore important factors affecting the Antarctic marine ecosystem. The objectives of the present study are to examine the circumpolar occurrence of the eugregarines parasitizing the Antarctic krill, and to evaluate the relationship between geographical locations and variation in eugregarine infection in krill.

Materials and methods

Antarctic krill were collected from Atlantic, Indian and Pacific sectors of the Southern Ocean (Fig. 1). Samples from the Atlantic sector were collected in the vicinity of the Antarctic Peninsula (A) during the 7th Antarctic Expedition from October 1994–March 1995 by the RV *Kaiyo-maru* of the Japan Fisheries Agency. Samples were collected with the KYMT net (Kaiyo-maru mid-water trawl: mouth area of 9 m^2, mesh aperture of 3.4 mm) (National Research Institute of Far Seas Fisheries 1995). Samples from the Pacific (P1-P4) and Indian (I1-I4) sectors were captured from October 1977 to February 1978 by the RV *Banshu-maru*. Samples were collected with the surface mid-water trawl (mouth area of 360 m^2, mesh aperture of 15 mm) (Japan Marine Fishery Resources Research Center 1978). Samples from near Syowa Station (S) in the Indian sector were collected with a parasol net from April to December 1982. These samples were immediately fixed in 10% buffered formalin seawater on board.

In the laboratory, about 20 individual krill were chosen randomly from each sample. Body length was measured from the anterior margin of the orbit to the tip of the telson in 1 mm size classes (Siegel 1982, Miller 1983). Maturity stage and sex were classified according to Makarov & Denys (1981), and they were grouped into five divisions: juvenile, sub-adult male, sub-adult female, adult male and adult female. The digestive tracts were removed, put on a glass slide, and torn to pieces with forceps. Two motile stages of the eugregarine, immature gamont and mature gamont stages as defined by Avdeev (1987) were then counted under an inverted microscope (Fig. 2).

The interaction between geographical location and maturity stage of krill was evaluated using an analysis of variance (ANOVA). Geographical locations were grouped

Table I. Percentage occurrence of *C. pacifica* and maturity stage of krill at each location in this study. Numbers in parenthesis indicate the total numbers of krill which had no eugregarines.

Station	No. of krill	Individual number of host krill					Prevalence (% krill infected)	Intensity of *C. pacifica* ± SD (range)
		Juvenile	Sub-adult		Adult			
			male	female	Male	female		
Pacific sector (P1-P4)	80	60 (4)	0	0	6	14	95.0	272.6 ± 985.3 (0–8089)
Indian sector (I1-I4)	80	0	13	14	12	41	100.0	553.6 ± 682.5 (4–2877)
Syowa Station	15	15 (1)	0	0	0	0	93.3	20.9 ± 22.0 (0–68)
Antarctic Peninsula	20	0	8 (2)	11	0	1	90.0	96.2 ± 103.5 (0–376)
Total	195	75 (5)	21 (2)	25	18	56	96.4	350.4 ± 787.8 (0–8089)

into four divisions; Pacific sector (P1-P4), Indian sector (I1-I4), Atlantic sector (A), and Syowa Station (S). Parasite counts were distributed approximately as a negative binomial, and every station had a variance greater than the mean, indicating an over-dispersed distribution. For comparison of variation in the abundance of eugregarines, the data was therefore transformed using a $\log_{10}$ function to reduce the bias of very high numbers. The goodness of fit was tested by the Chi-square test at a 5% level of significance. All statistical analyses were carried out using the Statistical Package for the Social Science (SPSS) for Windows, version 15.0.

The term "prevalence" as used in this paper is defined as the number of infected hosts by a particular parasite species divided by the number of hosts examined for that parasite species and the term "intensity" is defined as the number of individuals of a particular parasite species in a single infected host (Bush *et al.* 1997).

Results

All eugregarines in this study were *Cephaloidophora pacifica* (Fig. 2). They were found at all locations with 96.4% of prevalence, and showed a remarkably wide range in numbers (Table I). Prevalence of *C. pacifica* was 95.0% in the Pacific sector, 100.0% in the Indian sector, 93.3% in the Indian sector, and 90.0% in the Atlantic sector. Samples from the Indian sector consisted of both sub-adult and adult krill, while at the Antarctic Peninsula, sub-adults dominated. Samples from Syowa Station were composed of juvenile krill only. Intensity of *C. pacifica* ranged from 0 to 8089 ind host^{-1}; mean intensity (± SD) was 350.4 ± 787.8 ind host^{-1} (Table I). Intensities of more than 1000 eugregarines were found in 22 specimens, while only seven krill had no eugregarines. Intensity, range and mean ± SD at the locations where sub-adult and adult dominated were much greater than at the locations dominated by juveniles.

Krill body length varied between 25.8 and 56.5 mm (mean: 41.6 ± 8.9) with 41.5% females, 20.0% males and 38.5% juveniles. As the host matured, mean body length increased in both male and female and intensities of *C. pacifica* also increased (Table II). Statistical analyses revealed that the geographical location did not have a significant effect on intensity of *C. pacifica* ($P = 0.121$, Table III). On the other hand, the intensity of *C. pacifica* was significantly different at each maturity stage ($P < 0.001$, Table III).

Table II. Mean body length and intensity of *C. pacifica* at each maturity stages of krill.

Maturity stage	Number of krill	Mean body length of krill (mm) ± SD	Intensity of *C. pacifica* ± *SD*
Juvenile	75	32.3 ± 3.8	21.0 ± 44.4
Sub-adult male	21	45.9 ± 2.0	464.3 ± 592.7
Adult male	18	49.8 ± 4.7	505.1 ± 747.3
Sub-adult female	25	45.3 ± 4.6	396.6 ± 540.3
Adult female	56	48.3 ± 5.0	678.6 ± 1219.6

Table III. Interaction of geographical location and maturity stage of krill (details of ANOVA).

Source of variation	Degrees of freedom	Variance ratio	Significance
Location (A)	3	1.964	0.121
Maturity stage (B)	4	24.875	< 0.001
A × B	3	2.054	0.108

Discussion

Cephaloidophora pacifica occurred in *Euphausia superba* from all sites examined. Avdeev (1985) studied these parasites of *E. superba* in 1982 around Elephant Island and westwards to the east of the Indian sector, and during 1984 from the eastern part of Riiser–Larsen Sea (30ºE) to the middle of Sodruzhestva Sea (68ºE). Dolzhenkov *et al.* (1987) pointed out that areas of occurrence of the two eugregarinid species *C. pacifica* and *C. indica* were separated, and *C. indica* was found in *E. superba* only in the eastern part of the Indian sector (110–148ºE). However, in this study, all eugregarines found in the Indian sector (110–135ºE) were identified as *C. pacifica* which can therefore be considered as distributed throughout the Southern Ocean.

The present study on *C. pacifica* demonstrated that their distribution show the overdispersed pattern (i.e., the standard deviation is greater than the mean). This frequency distribution pattern is typically found in parasites

https://doi.org/10.1017/S0954102008001016 Published online by Cambridge University Press

living in marine species (Boxshall 1974, Tanner *et al.* 1980, Jakob 1987, Takahashi *et al.* 2004). Takahashi *et al.* (2004) suggested that this difference may be due to the body length and the maturity stage composition of hosts. Thus, larger krill have more eugregarines than smaller krill at the same maturity stage. Statistical analyses showed *C. pacifica* abundance increases with the maturity of the krill. On the other hand, the geographical location did not have a significant effect, thus the variation of intensity for each station may be primarily influenced by the stage compositions of the Antarctic krill.

The high prevalence of *C. pacifica* indicates that the relation between the eugregarines and Antarctic krill is strong. Takahashi *et al.* (2003) survey of Antarctic krill from the vicinity of the Antarctic Peninsula showed similar results, with 98.2% of krill ($n = 383$) being infected with *C. pacifica*. Avdeev (1985) reported that eugregarines were found in 75.0% of krill examined ($n = 1848$) in the Pacific and Indian sectors of the Southern Ocean. Avdeev & Vagin (1987) suggested that the eugregarines in the mid-gut gland have a pathological effect because of the close correlation between the number of eugregarines and clots in the diverticula of the mid-gut gland. A histological study of Kawaguchi (unpublished observations) revealed that the eugregarines cause damage to mid-gut gland and intestinal epithelium. Therefore, heavy infestation by eugregarines may have negative impacts on krill biomass and productivity. The results of the present study show that infestation of Antarctic krill by *C. pacifica* is a circum-Antarctic phenomenon, and it occurs throughout the Southern Ocean. It is important to take into account the ecological influences of eugregarine infestation on the Antarctic krill, due to the fact that krill is a key species of the Antarctic marine ecosystem.

Acknowledgements

We are grateful to Drs K. Hoshide, Yamaguchi University and S. Imai, Nippon Veterinary and Animal Science University, for their helpful information on the eugregarinid protozoans, S. Taguchi, Soka University and T. Kikuchi, Yokohama National University, for their valuable comments. We also thank the officers and crew of the RV *Kaiyo-maru* and the RV *Banshu-maru* for their assistance. This research was partially funded by the Sasagawa Scientific Research Grant from The Japan Science Society to Masaki Kobayashi.

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