

# *Petasaria heterolepis* (Prymnesiaceae) from the Southern Indian Ocean

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**ABSTRACT:** Sea surface and sub-surface (0-110m) water samples were collected from the Southern Indian Ocean between 39°S and 65°S (along ~48°E and 57.3°E) during the austral summer of 2010 which yielded *Petasaria heterolepis*. Out of the two known *P. heterolepis* forms, the scales with brim are observed with a size range (short and long axes length) of 1.68×1.80µm-2.17×2.27µm. The occurrence of minute pores on the scales is documented except on the brim which remains poreless. The elemental analysis of the scales revealed silica (Si) as the major constituent with the occurrence of a small peak of calcium (Ca). In spite of the presence of Ca in the scales, *P. heterolepis* showed lack of birefringence in the light microscopic observation. *P. heterolepis* is neither recognizable in the fossil record nor in the bottom sediments and shows affinity towards the extant *Prymnesium neolepis* recorded from off the Japan coast and Southern Indian Ocean. The present study highlights the occurrence of *P. heterolepis* in the open ocean regions of the Southern Indian Ocean stressing its ability to grow under diverse oceanographic conditions. The phylogenetic-molecular aspects need to be carried out which could reveal better understanding of rare Si and Ca accumulation among such haptophyte algae.

Keywords: *Petasaria heterolepis*, Siliceous haptophyte, Southern Indian Ocean, Silica, Calcium

## INTRODUCTION

The Haptophytes (Prymnesiophyta) are the most important marine phytoplankton group which first appeared in the geological record about 230 million years ago (Jafar 1983) and in the modern ocean they play a significant role as a primary producer (Graham and Wilcox 2000). On account of their wide ranging occurrence and fast turnover rates, they are primary harvesters of solar energy, control climate and form a part of key biogeochemical cycles in the ocean (Staat et al. 2000; Thierstein and Young 2004; de Vargas et al. 2007). Among the known marine haptophytes, most species possess fibrillar unmineralized body scales and many possess calcified scales with unmineralized organic layer underneath known as coccoliths (Jordan and Chamberlain 1997; Young et al. 1997).

In marine ecosystem corals, foraminifera and coccolithophores are recognized as the calcifying taxa whereas; Synurophyceae, Cryosphyceae, Dictyocnophyceae and Bacillariophyceae are known for their silicification (Preisig 1994; Preisig et al. 1994). The siliceous scale is very rare in the haptophytes and has previously been reported in the cysts of *Prymnesium parvum* (Hibberd 1980). A haptophyte alga containing siliceous scales, *Prymnesium neolepis* was first discovered by Yoshida et al. (2006) from the surface and subsurface water samples collected from Shiribeshi seamount off Japan coast and later documented by Patil et al. (*in press*) from the Southern Indian Ocean. Based on the occurrence of silica in the haptophytes the previous belief that the heterokonts silicify and haptophytes calcify is not acceptable at all times (Yoshida et al. 2006).

*Petasaria heterolepis* a haptophyte with conspicuous scale was first described by Moestrup (1979) from the seawater samples collected from Kaikoura, New Zealand (42.25°S; 173.42°E)

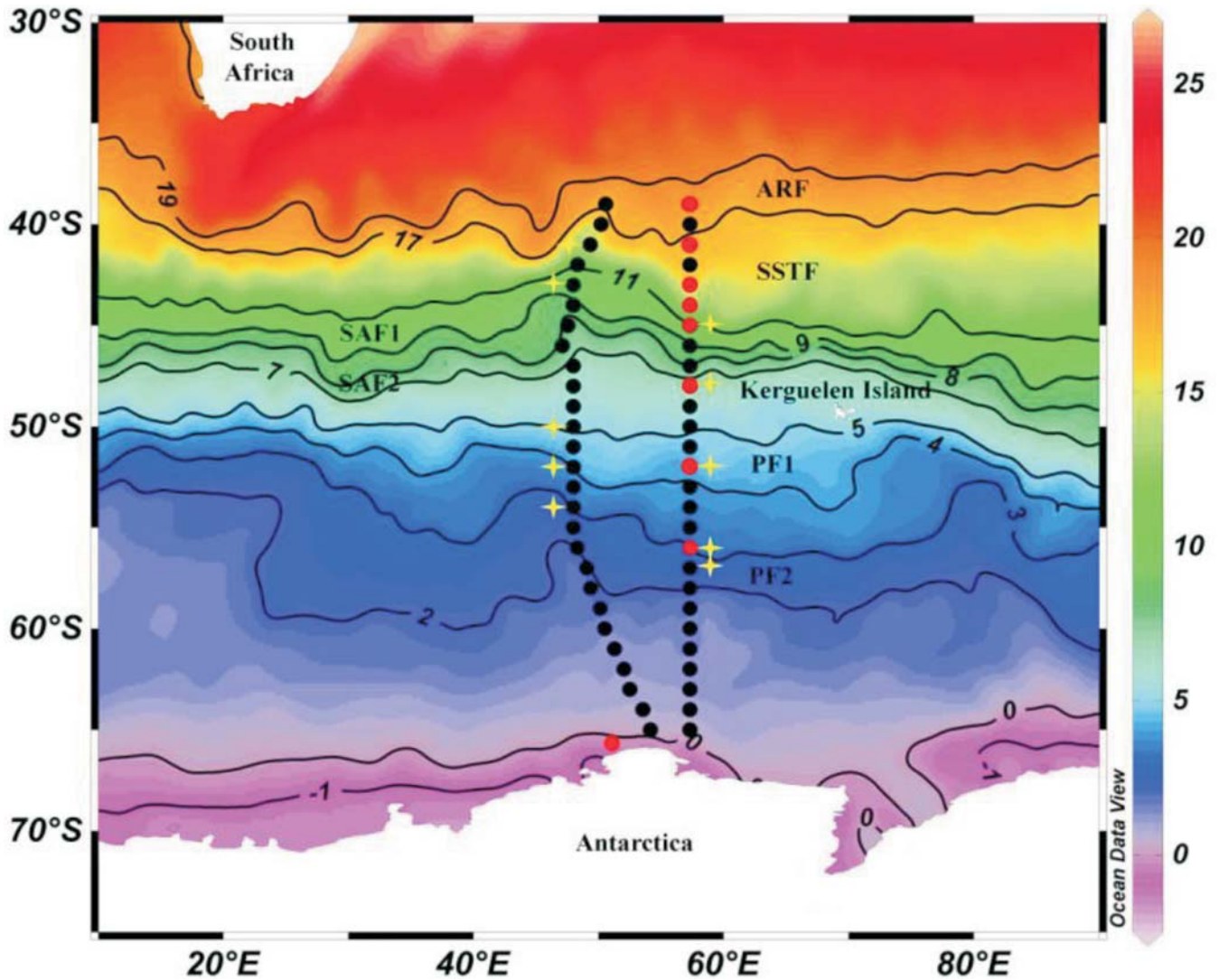
and later by LeRoi and Hallegraeff (2006) from southern Tasmanian coastal waters (43.08°S, 147.22°E; 42.46°S, 147.33°E and 43.02°S, 147.56°E). The *P. heterolepis* is only documented previously from whole mount preparations with suspicious presence of chloroplast (Patterson and Zölffel, 1991). Nishida (1979) mentioned this species as “species indetermined” previously in the atlas of Pacific nannoplankton (pl. 22, fig. 4). In the present study, we document a siliceous scale bearing *P. heterolepis* from the open oceans regions of the Southern Indian Ocean in the surface and subsurface waters during austral summer of 2010 (January-February 2010).

## MATERIALS AND METHODS

### Field Sampling

#### *Southern Indian Ocean along 57.3°E and 48°E; between 39°S to 65.49°S*

Sea surface and vertical profile samples (0, 20, 40, 60, 80 and 110m) were collected from the southern Indian Ocean during the austral summer of 2010 (January-February, 2010) aboard ocean research vessel *Sagar Nidhi*. The cruise track followed during the study period is shown in Fig. 1 and the locations of *P. heterolepis* occurrence with associated physico-chemical parameters are given in Table. 1. A total of 46 sea surface and 54 water samples from 9 vertical profile stations were collected across the oceanic frontal regions [Agulhas retroflection Front (ARF), Southern Subtropical Front (SSTF), Subantarctic Front (SAF1, SAF2) and Polar Front (PF1, PF2)] of the Southern Indian Ocean. The sea surface and vertical profile samples were collected using 5l capacity Niskin bottles attached to a rosette. The temperature and salinity profiles were obtained using Conductivity Temperature Depth (C.T.D.) sensors attached to a ro-



TEXT-FIGURE 1  
 Showing sea surface (Black dots) and vertical profile (Red dots, 0-110m depth) sampling locations in the Southern Indian Ocean. showing recovery stations for *Petasaria heterolepis*. Location of fronts after Anilkumar et al. 2006, Luis and Sudhakar 2009. ARF, Agulhas Retroflection Front; SSTF, Southern boundary of Subtropical Front; SAF1, SAF2, Subantarctic Front; PF1, Polar Front1; PF2, Polar Front2. SST image generated using data obtained from, PO.DAAC LAS v7.3 Live Access Server (<http://thredds.jpl.nasa.gov/las/getUI.do>)

sette. The nutrients (nitrate, nitrite, phosphate and silicate) analysis was carried out immediately aboard using Shimadzu UV-1800 spectrophotometer following standard protocols described by Grasshoff (1983).

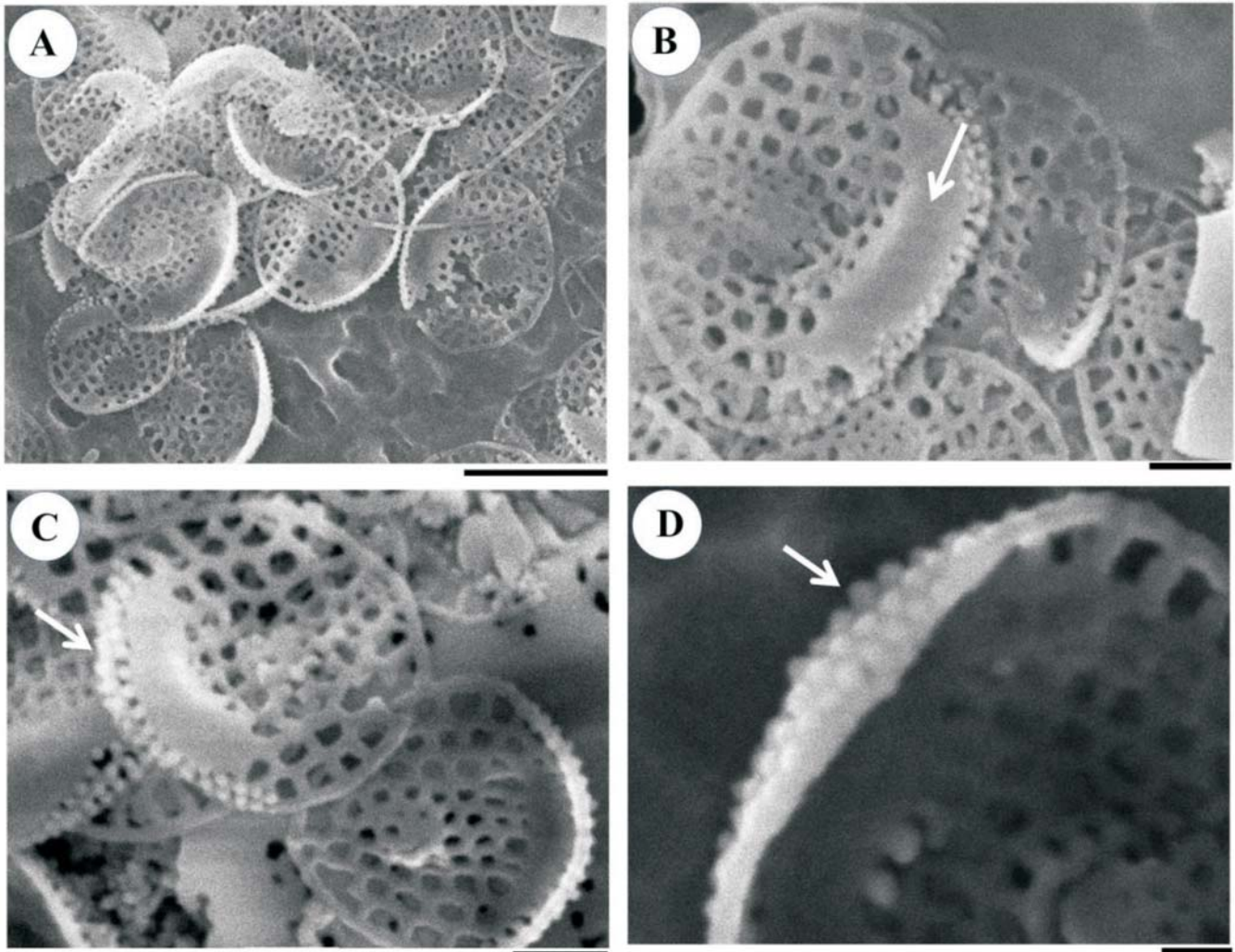
**Preparation of samples for SEM**

One liter seawater sample was pre-filtered through a 63 micron mesh to remove large planktons. This pre-filtered water was then passed through Nuclepore Trek-Etched membrane filters of 0.2µm pore size, 47mm diameter, using Pall manifold filtration unit under low vacuum. The filter papers were further transferred into plastic petri-dishes and oven dried at 40°C for 48 hours and stored in the sealed plastic petri-dishes until analysis.

***Petasaria heterolepis* evaluation**

About 5mm<sup>2</sup> filter paper was cut and placed on a double sided carbon tape attached to a 1cm diameter aluminum stub. The mounted filter paper was further sputter coated with platinum (approx. 2nm thickness) and 200 fields of views were observed at 3000× magnification using Scanning Electron Microscope (SEM) JEOL 6360 LV at 5-15 Kv acceleration voltage. The *P. heterolepis* were magnified and micrographs images were stored for records. The stub with abundant *P. heterolepis* has been archived in the SEM-EDS lab with an archival number SEM-EDS Lab/Arch-01/*P. heterolepis*. The X-ray elemental analysis was performed using Oxford Energy Dispersal Spectrometer (EDS) to study the composition of *P. heterolepis* scales.





TEXT-FIGURE 2

Scanning Electron micrographs of *Petasaria heterolepis* observed in the Southern Indian Ocean. **A.** collapsed cell body on the filter paper. **B.** raised poreless brim, **C, D.** minute elevations on outer surface of raised rim. Scale- **A**= 2 $\mu$ m; **B,C**= 0.5 $\mu$ m; **D**= 0.2 $\mu$ m. (Sample- 52°S, 48°E).

#### Electron and light microscopic observation of *P. heterolepis* (carried out using 2 $\mu$ m pore size filter paper)

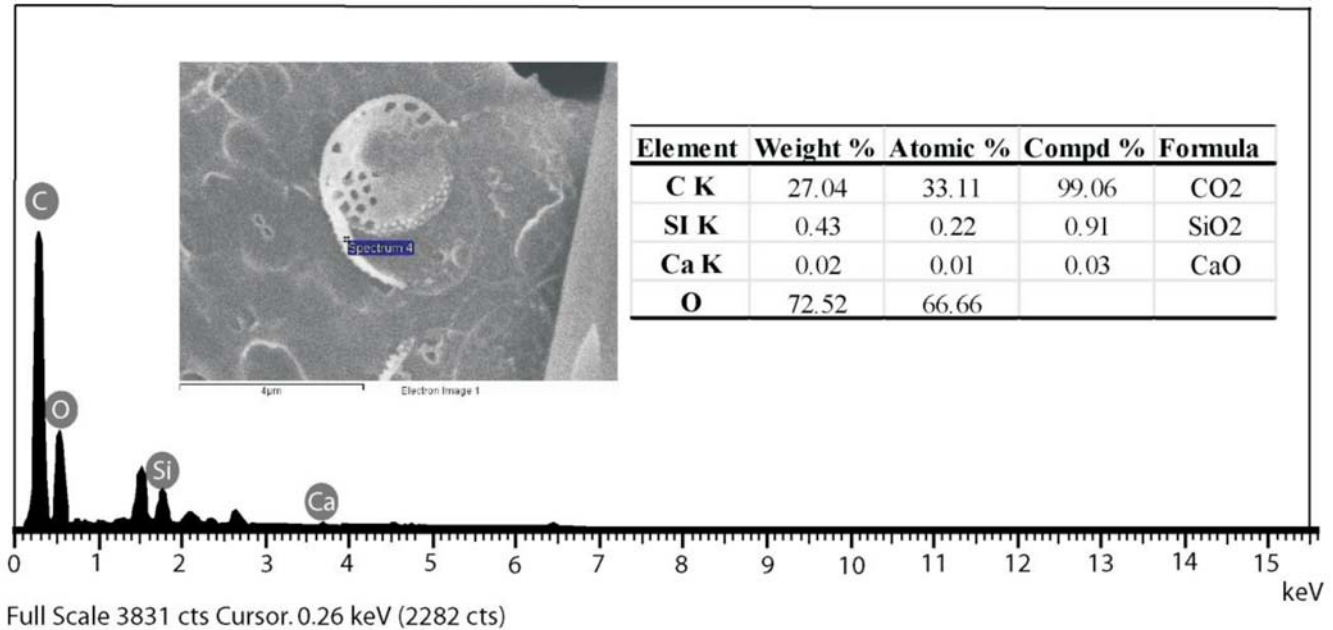
For electron-light microscopic observation of *P. heterolepis*, a small filter paper was mounted on a coverslip, placed on a 1cm diameter Aluminum stub, sputter coated with approx. 2nm thickness platinum and observed under SEM. The position of *P. heterolepis* was noted down based on the coordinates of filter paper pores and associated coccolithophores around. The coverslip containing filter paper was removed from the stub, mounted on a glass slide and observed under Olympus BX 51 polarizing research microscope. The exact position of the specimen was identified and verified with the help of associated coccolithophores present around *P. heterolepis* and with the help of filter paper pores.

#### RESULTS

While studying the vertical and lateral distribution of coccolithophores from the southern Indian Ocean during the austral summer of 2010 (January-February, 2010), *P. heterolepis*, a protist consisting wide brimmed hat shaped scales were detected from the samples collected between 41°S-57°S (along ~48°E and 57.3°E). The X-ray elemental analysis of *P. heterolepis* scales observed in the study period was carried out that revealed, Si as the major constituent of these scales.

#### Previous report

Coastal waters of New Zealand, Red Sea, Denmark (Moestrup, 1979) and Southern Tasmanian waters (LeRoi and Hallegraef, 2006).



TEXT-FIGURE 3

The elemental composition of scales of *Petasaria heterolepis* (43°S, 48°E, surface) collected at the Southern Indian Ocean.

Family PRYMNESIACEAE Conrad ex O.C. Shmidt 1931  
Genus *Petasaria* Moestrup 1979

***Petasaria heterolepis*** Moestrup 1979  
Text-figure 2a-d

**Remarks:** The scales measure, *P. heterolepis* short axis- 1.68×1.80µm and long axis- 2.17×2.27µm, with a circular or oval-rectangular base. Scales shaped like a wide-brimmed hat, comprised of mainly silica, part of the base is patternless with raised rim on one side, and other area lacks a raised rim. Each scale carries hollow projections of uneven diameter; perforated area near scale centre; no pores in wide brim; towards the side cells consists of elevations those look like short spines which are neatly arranged in rows (text-fig. 2 C,D).

In the present study, collapsed cell body (6.7-7.7µm after breakup) and individual siliceous scales were observed in the dried filter papers. No definite cell shape is described and well documented “Spider Web” organic scales (Moestrup, 1979, Fig. 65), were not found in this study. Such organic scales could be contamination, as they were also not observed in partly preserved specimen of *P. heterolepis* from Gulf of Elat (Moestrup, 1979, Fig. 66). A single long flagellum seen in complete specimens documented by Moestrup (1979, Figs. 61-62) is interpreted as closely resembling haptonema of *H. neolepis* and hence the taxonomic assignment of this monospecific genus to Haptophyta remains questionable and provisional (Yoshida et al. 2006).

**Present finding of *Petasaria heterolepis***

*Petasaria heterolepis* was observed in the southern Indian Ocean at surface and subsurface waters (0-80m depth) between 41°S-57°S along ~48°E and 57.3°E longitude (Fig.1). The max-

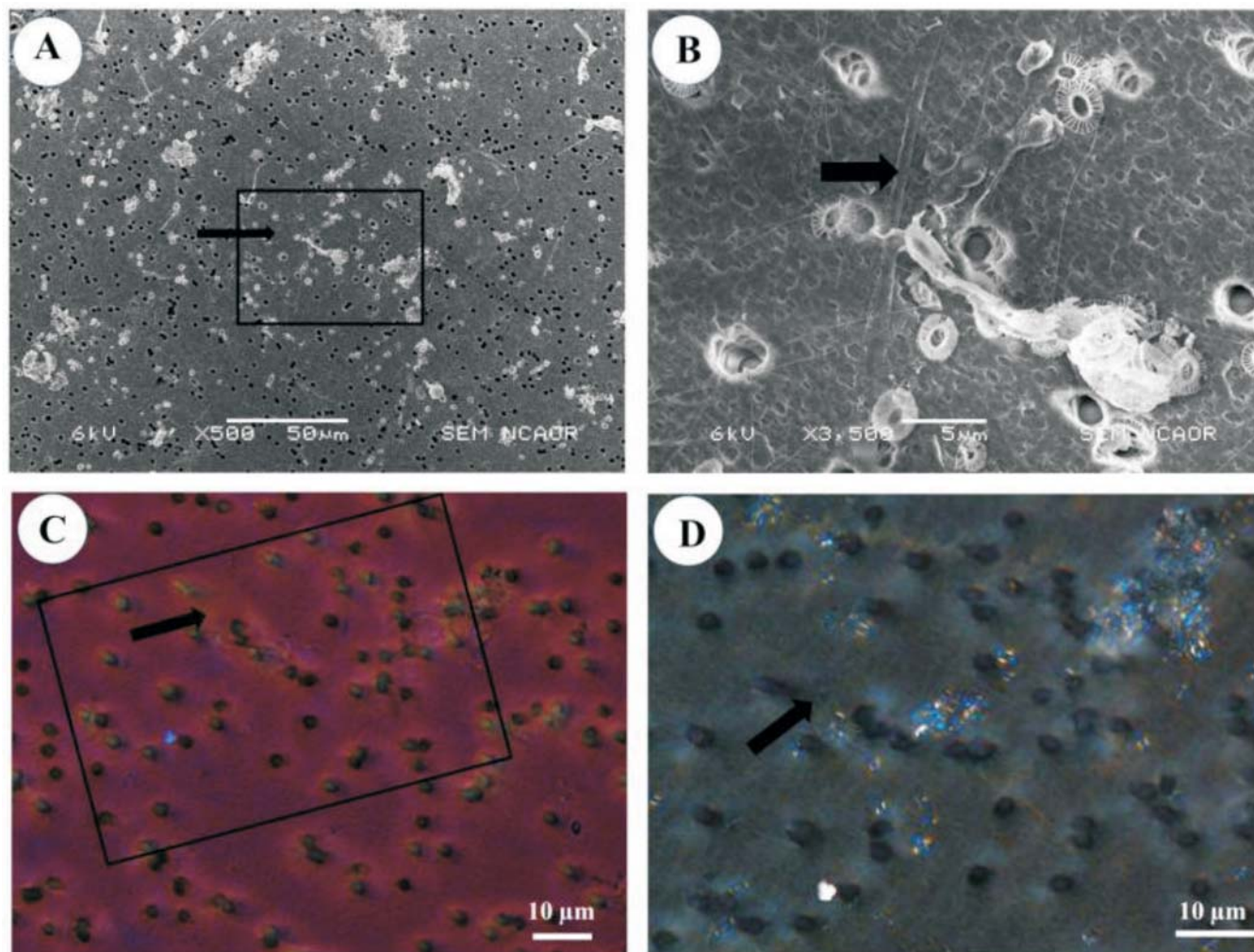
imum density of this species observed was 3.11×10<sup>5</sup>/l in the sea surface water sample collected at 43°S, 48°E.

**DISCUSSION**

We have documented presence of *P. heterolepis* with scales containing wide-brimmed hat from the open ocean region of the southern Indian Ocean. In this study, the scales of *P. heterolepis* measured 1.68×1.80µm-2.17×2.27µm which are smaller than those documented by Moestrup (1979) (1.7×1.9µm-2.9×3.2µm) but larger than those documented by LeRoi and Hallegraef (2006) (1.5×1.6µm-1.8×1.9µm). The elemental analysis of *P. heterolepis* in the present study shows that silica is a major component of these scales. In the histogram of elemental analysis a small peak of Ca is observed, akin to the observations of Moestrup (1979) indicating presence of silica as well as minor Calcium in the scales of *P. heterolepis* (text-fig. 3, Table. 2).

In spite of the occurrence of little peak of Ca in the X-ray elemental analysis, during the routine light polarized microscopy, *P. heterolepis* scales were not visible and remains undetected owing to its extremely low relief (Fig. 4). The two haptophyte species, *P. heterolepis* and *P. neolepis* show similarity in the elemental composition where Si is the major component of these scales. The observed occurrence of *P. heterolepis* as far as 57°S in the southern Indian Ocean shows its ability to grow in the open ocean waters of southern Indian Ocean. The ultrastructure changes in the wide brimmed-hat shaped scales of *P. heterolepis* as well as their elemental analysis needs to be studied in detail. The protists containing Si as well as minor Ca in scales occurring in the Southern Indian Ocean, most probably suggests the initial signature of adapting itself to the changing Southern Ocean and expanding its presence towards southern latitudes. The change in ocean sea surface temperature and nutrient concentrations may considerably contribute to its fast turnover rates aiding in primary biomass production. In this study the elevated abundance of *P. heterolepis* observed in the SAF2 and PF1 re-





TEXT-FIGURE 4

Polarized light- and scanning electron microscopic differentiation of *Petasaria heterolepis* collected from the Southern Indian Ocean. **A,B.** Scanning Electron micrographs showing its position (arrow) **C,D.** Under crossed polarized illumination, specimen (arrow) shows lack of birefringence and remains dark, while coccolithophores are birefringent.

gion of the Southern Ocean indicates its preference to cold, high silicate containing open ocean waters.

The precise cause of silicification in haptophytes is not yet clear (Yoshida et al. 2006). It is quite feasible that the formation of siliceous scale in haptophytes is homologous and partly orthologous to the machinery used in the calcification of haptophytes demanding further research (de Vargas et al. 2007). The polycondensation of  $\text{Si}(\text{OH})_4$  for cell wall formation is energetically favorable compared to calcium or cellulose synthesis where, only 2% of the cellular energy is required to provide 20% of the cell weight (1ATP is required to take up every silicon and deposit) (Raven, 1983) that indicates major evolutionary advance compared to calcification. It has also been reported that the adaptation of non-calcifying haptophytes possess the ability to switch from autotrophic to heterotrophic mode of nutrition and vice versa; with abundant nutrients they lead an autotrophic life and with low nutrient and some unknown factor, they could survive on ingesting prey (Medlin et al. 2008).

In the southern Indian Ocean, siliceous frustules in haptophytes, are possibly responsible for the shift of mineralization strategy from carbonate to siliceous and make the altered structure to cope with adaption to low temperature, high nutrient conditions.

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TABLE 1

Temperature (°C), salinity (psu) and nutrient composition (µM) of sampled locations in the Southern Indian Ocean where *Petasaria heterolepis* was documented.

Latitude (°S)	Longitude (°E)	Sample Depth (m)	Station depth (m)	<i>P. heterolepis</i> abundance × 10 <sup>5</sup> nos/l	Temperature (°C)	Salinity (psu)	NO <sub>2</sub> (µM)	NO <sub>3</sub> (µM)	PO <sub>4</sub> (µM)	SiO <sub>4</sub> (µM)
45	57.3	40	5582	0.031	11	34.02	0.13	13.17	0.93	4.01
48	57.3	0	4223	0.031	7	33.71	0.11	10.91	1.77	3.51
48	57.3	80	4223	0.031	6.5	33.73	0.23	19.34	1.6	5.27
52	57.3	20	4776	0.37	4	33.87	0.23	22.5	1.73	13.92
52	57.3	40	4776	0.18	3.5	33.88	0.15	23.21	3.82	13.86
56	57.3	40	4094	1.12	2.5	33.94	0.17	19.33	1.85	17.69
57	57.3	0	4670	0.062	3	32.38	0.16	17.89	1.51	14.30
41	49.36	0	2821	0.031	13	34.09	0.21	9.28	1.11	2.20
43	48	0	2732	3.11	8.5	32.8	0.29	20.62	1.25	5.02
50	48	0	3291	0.031	5.5	32.51	0.18	17.47	1.56	4.08
52	48	0	5449	0.93	4.5	33.74	0.35	19.30	1.29	6.27
54	48	0	5321	0.093	4	33.73	0.35	19.07	1.74	11.16

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TABLE 2

Range of elemental composition recorded in *Petasaria heterolepis* (obtained from 5 different areas) which was collected from Southern Indian Ocean.

Element	Weight %	Atomic %	Compd %	Formula
<b>C K</b>	26.87-27.15	33.11-33.21	98.46-99.5	CO <sub>2</sub>
<b>SI K</b>	0.24-0.7	0.12-0.37	0.5-1.5	SiO <sub>2</sub>
<b>Ca K</b>	0-0.03	0-0.01	0-0.04	CaO
<b>O</b>	72.4-72.61	66.66-66.67		

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