

A re-examination of the type material of *Entomoneis paludosa* (W SMITH) REIMER and its morphology and distribution in African waters

Tatenda DALU^{1*}, Jonathan C. TAYLOR^{2,3,4}, Nicole B. RICHOUX¹ & P. WILLIAM FRONEMAN¹

¹ Zoology and Entomology, Rhodes University, P O Box 94, Grahamstown 6140, South Africa; *Corresponding author e-mail: dalutatenda@yahoo.co.uk

² School of Biological Sciences, North-West University, P Bag X6001, Potchefstroom 2520, South Africa

³ South African Institute for Aquatic Biodiversity (SALAB), P Bag 1015, Grahamstown 6140, South Africa

⁴ Botanic Garden Meise, Nieuwelaan 38, 1860 Meise, Belgium

Abstract: The current study aims to enhance the understanding of the distribution and morphology of the diatom *Entomoneis paludosa* W SMITH 1853 in African waters. The type material of *Entomoneis paludosa* (W SMITH) REIMER was examined using light and scanning electron microscopy and the morphological characters were compared with new specimens sampled from a temperate river in South Africa. The wider distribution of this taxon on the African continent is discussed, and its relationship to water quality variables.

Key words: benthic, distribution, *Entomoneis paludosa*, Kowie system, morphology

INTRODUCTION

There are currently 44 species and infraspecific taxa in the genus *Entomoneis* (family: Entomoneidaceae) in the Integrated Taxonomic Information Systems, National Center for Biotechnology Information taxonomy and World Register of Marine Species database at present, of which 16 have been flagged as currently accepted taxonomically including *Entomoneis paludosa* (W SMITH) REIMER (SCOTT & THOMAS 2005). The original publication and holotype designation of *Entomoneis paludosa* was by W SMITH (1853) as *Amphiprora paludosa* and was later transferred to *Entomoneis* by Reimer in 1975 (SALVADOR in GUIRY & GUIRY 2014). To our knowledge, no study of William Smith's type material has since been carried out.

The diatom *Entomoneis paludosa* is an epipellic diatom that occurs in streams with elevated salinity, and there have been reports of this species occurring in standing waters with elevated electrolyte concentrations (BAHLS 2012). Little information is known on the present distribution and morphology of this species on the African continent, and therefore this paper aims to contribute towards the knowledge base in terms of distribution and morphology of *E. paludosa*, and to compare the morphological characteristics of the cells from our samples to those found in the type material. We discuss *E. paludosa* var. *subsalina* (CLEVE) KRAMMER and postulate that it may be con-specific with the nominate variety but to confirm this there

will need to be further studies of Cleve's type material as well as morphological and molecular studies of discrete populations. This is unfortunately outside the scope of the present study.

MATERIALS AND METHODS

Study area. The Kowie River is a permanently open temperate system draining a relatively small catchment area of approximately 800 km² (HEYDORN & GRINDLEY 1982; WHITFIELD et al. 1994). The Kowie River is classified as intermediate and its source is the Cape Fold Belt Mountains. Its major tributaries are the Bloukrans, Brakrivier and Lushington (or Torrens) Rivers (HEYDORN & GRINDLEY 1982). Epipellic diatom samples were collected along the Kowie system, Eastern Cape, South Africa (33°21'57.7"S–33°32'23.6"S, 026°37'38.0"E–026°48'13.0"E), from seven study sites over four periods: September 2012, December 2012, February 2013 and May 2013 (Fig. 1). Freshwater sites F1 and F2 were polluted with sewage draining from Grahamstown.

Diatom sampling and processing. The benthic habitat in the Kowie River was dominated by clay/silt (55%) and cobbles or rocks (45%) with decaying detritus. The submerged macrophyte *Cyperus eragrostis* LAMARCK covered most of the littoral zone (60%), with *Phragmites australis* (CAVANILLES) TRINIUS ex STEUDEL covering 15% of the total littoral zone surface area in the river section while *Schoenoplectus brachyceras* (HOCHSTETTER ex A RICHARD) LYE (53%) and *Spartina maritima* (CURTIS) FERNALD (32%) were the dominant macrophytes in the estuary (DALU et al. 2014). Epipellic diatom samples were collected in 500 ml

containers from the Kowie system. Samples were digested in potassium permanganate and hydrochloric acid and mounted in Pleurax (r.i. 1.73), for details see TAYLOR et al. (2005). After sample preparation an aliquot was dried onto a glass microscope coverslip, coated with gold palladium and viewed using an FEI Quanta FSEM electron microscopy housed at the laboratory for Electron Microscopy, North-West University. Light microscope (LM) studies were carried out using a Nikon 80i compound light microscope, equipped with Normarski DIC and using a 100× 1.45 N.A. objective. Valve width and length of specimens were measured in micrometres (µm). The density or abundance of *E. paludosa* was determined by counting the numbers present in five 10 ml sub-samples and the mean value was recorded. Species abundances (densities) were presented as number per litre.

William Smith's diatom type material, sample collection locality Lewes, England, was obtained from the collection of the Botanic Garden Meise in Belgium. Material dried on mica was stored in small folded envelopes mounted on sheets. The envelope was marked in Smith's own hand 'near Lewes, September 1852', exactly matching the published description of the type locality. No *E. paludosa* var. *subsalina* type material was examined. Light microscope (LM) studies were of a section of the original mica preparation made by Smith, and to prevent disturbance of the integrity of the sample the mica was left intact and the cells photographed using an Olympus BX51 light microscope and colour view camera without additional cleaning or mounting in a resin. Although striae may not be observed in this manner, the shape, size and key features of cell morphology are visible. An additional very small section of mica was mounted on an aluminium stub, coated with gold palladium and examined under scanning electron microscope (SEM).

Physico-chemical factors sampling and analysis. The parameters of temperature, pH, dissolved oxygen, conductivity, salinity, total suspended solids, sodium chloride, resistivity and water transparency were measured using portable probes (CyberScan Series 600, Eutech Instruments, Singapore). Flow velocity was measured using a portable flow meter (Flo-mate Model 2000, Marsh McBirney, Maryland). Water samples were collected using 500 ml polyethylene bottles for further laboratory analysis of phosphate, nitrate and ammonia using standard methods for examining water and wastewater (EATON et al. 2005).

Data analysis. We analysed locality and demographic data from the earliest available records to the present for *Entomoneis paludosa* in Africa. We undertook systematic literature searches, unrestricted by publication year, using the terms '*E. paludosa*', '*Amphiprora paludosa* var. *subsalina*', '*Amphiprora paludosa*', '*Amphicampa paludosa*', '*Amphiprora paludosa* var. *africana*', '*Amphiprora venusta*', '*Amphiprora paludosa* var. *pokornyana*' and '*Amphitropis paludosa*' in the academic search engines Scopus, Google Scholar and Web of Science. We reviewed reference lists of papers mentioning the species and searched peer reviewed African and international aquatic journals including African Journal of Aquatic Science, African Journal of Marine Sciences, African Journal of Ecology, Bibliotheca Diatomologica, Botanica Marina, South African Journal of Science, Diatom Research, Journal of Applied Phycology, Hydrobiologia, Nova Hedwigia, Revista de Biologia, Transactions of the Royal Society of South Africa, West

African Journal of Ecology, Water SA and Zoologica Africana. We reviewed, where accessible, expedition reports, checklists and project reports from National Museums.

Entomoneis paludosa density values were log (x+1) transformed to stabilise the variance (DALU et al. 2013). To determine whether to use linear or unimodal methods for the analysis, detrended canonical correspondence analysis (DCA) was employed. The gradient lengths were examined and since the longest gradients were between 3 and 4, a unimodal model RDA was selected (LEPŠ & ŠMILAUER 2003). RDA was performed on the *E. paludosa* datasets to examine the relationships between *E. paludosa* taxa and selected physico-chemical variables using 999 Monte Carlo permutations at $p < 0.05$ significance level in Canoco version 4.5 software (TER BRAAK 2002).

Multiple regression analysis was carried out using log (x+1) transformed data to evaluate the relationships that existed between the physico-chemical factors and *E. paludosa* species abundances. A non-parametric Kruskal-Wallis test was used to test for differences in physico-chemical factors among sites and time periods after the data was found to violate several ANOVA assumptions tested using Shapiro-Wilk test. SPSS version 16.0 was used for statistical analysis (SPSS INC. 2007).

RESULTS

Entomoneis paludosa potential distribution

To determine the distribution of this taxon in Africa, we found 36 locality records, 29 (81%) from published sources and 7 (19%) from National Museum records databases. *Entomoneis paludosa* was mostly identified from sites near the coastline (Table 1). Most of the records were from southern Africa 18 (50%), the least from east Africa 2 (8%) and one whose exact locality could not be identified in the records (Table 1).

Morphological characteristics

Phylum: Ochrophyta

Class: Bacillariophyceae

Order: Surirellales

Family: Entomoneidaceae

Genus: *Entomoneis*

Entomoneis paludosa (W SMITH) REIMER

Basionym: *Amphiprora paludosa* W SMITH

Homotypic synonyms: *Amphicampa paludosa* (W SMITH) RABENHORST 1864, *Amphitropis paludosa* (W SMITH) RABENHORST 1868

SMITH'S (1853) description was based on observations of cells with lengths between 40.6 and 106.7 µm, and 23.6 striae in 10 µm. Our examination of the type material showed a greater range in striae values from 22–26 in 10 µm. Light microscope images from the type mica are presented in Figs 2–8 and SEM images in Figs 9–20. Figs 2 to 8 show a rather high variability in valve shape, especially with respect to the junction between the keel and the surface of the valve. When

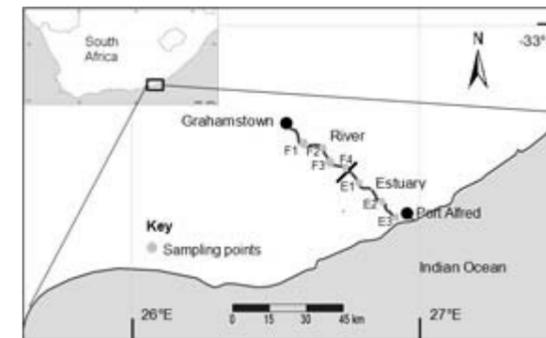


Fig. 1. Location of the sampling points on the Kowie system, Eastern Cape (South Africa).

viewed with SEM, a number of key features become visible. Costae extend from the valve mantle to the raphe canal and separate striae are composed of single rows of areolae (Fig. 13). These areolae are occluded on the outside by hymenes (Fig. 13) and are open on the inside (Fig. 14). The girdle bands are perforated by large elongate areolae occluded by cribra (Fig. 15). The raphe is housed in a tubular canal and the distal ends are slightly deflected (Fig. 16), the central endings are widened and drop-shaped (Figs 17 and 18) and may or may not be sunken in between silica ridges (Figs 17 and 18). The raphe is housed in a tubular canal which is perforated by small areolae occluded on the outside and open on the inside (Figs 19 and 20).

Scanning electron and light microscope micrographs of a single population of *E. paludosa* from the Kowie River system, along with data derived from CHOLNOKY (1966a, b), GIFFEN (1970) and BAHLS (2012), were used to facilitate the elucidation of the morphology of *E. paludosa*. The length range of *E. paludosa* in samples from the Kowie River was 28.5–91.5 µm, with a valve width range of 7.8–9.3 µm excluding the keels (Figs 21 to 35). The junction line which lies between the valve and keel contains a single bulge that is smoothly rounded and flattened in large specimens (Fig. 35), with that of smaller specimens being more angular (Figs 21 and 24). In valve view (Figs 32 and 39), valves at the valvar plane are linear-elliptic to linear lanceolate with slightly concave and apiculate apices. In girdle view (complete frustules), diagonally opposing lobes lie at approximately the same level of focus, with the lobes flattened and rounded in large and small specimens, respectively. The striae, 21–26 in 10 µm, on the valve face continue onto the keel (Fig. 43), with the areolae on the valve face being very fine and numbering 18–25 in 10 µm within a striae. The keel is strongly marked off from the valve by a basal line that is made conspicuous by striae of the keel structure. The frustules usually come to rest in girdle view, dominated by two highly arched bilobate keels, one on each valve. Areolae on the keel are much more prominent and number 22–40

in 10 µm within a stria. The lobes are not all in focus due to fact that the keels are slightly torsioned. Several (approximately 4 or more) girdle bands are present that form a crossing, sigmoidal pattern (Figs 21 to 27).

Physico-chemical variation and diatom ecology

The physico-chemical conditions in which the species was found are highlighted in Table 2. Conductivity, total dissolved solids (TDS), salinity, ammonia and water depth increased downstream while phosphate decreased from upstream to downstream. Nitrate, temperature and pH generally fluctuated along the river-estuary continuum (Table 2). Significant differences ($p < 0.05$) were observed in TDS, salinity, resistivity, water depth, ammonia, conductivity and pH among the study sites while monthly significant differences ($p < 0.05$) were observed in temperature and water velocity. Using multiple regression, we found that *E. paludosa* abundance was significantly negatively correlated ($p = 0.027$, $R = -0.43$) with resistivity while significantly positively correlated ($p = 0.007$, $R = 0.51$) with pH (Figure 48). All other physico-chemical factors were not significantly correlated ($p > 0.05$) with *E. paludosa* abundances.

No *E. paludosa* specimens were recorded in the sites F1 and F2 (September) and F1 (February). The highest abundances (mean of 1206 l⁻¹) were recorded in May. The estuary sites (mean 656 l⁻¹) generally had a high numbers of *E. paludosa* compared to the river sites (mean 374 l⁻¹). After the October–November floods, there was a slight increase in the population abundance at each site.

Two physico-chemical variables (nitrates and salinity) were important in structuring *E. paludosa* taxa sample/sites (Fig. 49). The first and second axes of RDA explained 72.1% of the *E. paludosa* taxa sample/sites variance, and 94.1% of the environment–species variation. The sum of the first and second axis canonical eigenvalues was 0.72. The polluted freshwater sites F1 and F2 were associated with nitrate while estuary sites E2 and E3 were associated with salinity (Fig. 49).

DISCUSSION

Taxonomic considerations

In this paper we consider, based on our observations of type and other material, *E. paludosa* var. *subsalina* (CLEVE) KRAMMER as a possible synonym of *E. paludosa* var. *paludosa* (W SMITH) REIMER. CLEVE based his original description of the variety on his observations of cells with 23 striae in 10 µm. Later, HUSTEDT (1930) stated that the differences between the nominate variety and *E. paludosa* var. *subsalina* were found in the greater number of striae and in the angle of the junction of the valve face and keel.

Table 1. Historical *Entomoneis paludosa* (W. Smith) Reimer locality records in different geographic regions of Africa obtained from National Museums.

Year	Locality name	Source
1930	Unknown	CLEVE-EULER & TEIL (1930)
EAST AFRICA		
1898	Congo	LEUDUGER-FORTMOREL (1898)
1986	East Africa	GASSE (1986)
NORTH AFRICA		
1913	Mediterranean Sea	DE TONI & FORTI (1913)
1954	Sidi Bou Rhaba; Dayèt er Rousini (Morocco)	GAYRAL (1954)
1954	Lake Mariut (Egypt)	JOB (1954)
1980	Alexandria Harbour (Egypt)	HAEIMETAL (1980)
SOUTHERN AFRICA		
1947	Goedgedacht, Zoutpansberg, Namaqualand (South Africa) - Fossil	KENT & RODGERS (1947)
1960	Mtubatuba-Mtunzini (South Africa)	CHOLNOKY (1960)
1963	Eastern Cape littoral (South Africa)	GIFFEN (1963)
1960	Gulu River and the Yarra Stream (South Africa)	GIFFEN (1963)
1960	Swartskops River, Berg River, Piketberg (South Africa)	CHOLNOKY (1960)
1962	Malmesbury, Cape Town (South Africa)	CHOLNOKY (1962)
1963	Indian Ocean (South Africa)	WOOD (1965)
1966	Welkom, Freestate (South Africa)	CHOLNOKY (1966c)
1966	Botswana	CHOLNOKY (1966b)
1966	Nambia	CHOLNOKY (1966a)
1968	Santa Lucia Estuary (South Africa)	CHOLNOKY (1968)
1966	Indian Ocean (South Africa)	TAYLOR (1966)
1970	Kowie system (South Africa)	GIFFEN (1970)
1970	Mozambique	SOURNIA (1970)
1975	Swartskops Estuary (South Africa)	MASSON & MARIAS (1975)
1982	Seychelles and Mauritius	COTZE & RICARD (1982)
1983	Sundays and Great Fish Rivers (South Africa)	ARCHIBALD (1983)
2012–2013	Kowie River (South Africa)	present study
2014	Mdloti River, near coastline north of Umhlanga (South Africa)	Own samples (J.C. Taylor)
WEST AFRICA		
1898	Sierra Leone, Cameroon	LEUDUGER-FORTMOREL (1898)
1913	Tripoli (Sierra Leone)	DE TONI & FORTI (1913)
1956	Dakar (Senegal)	SILVA (1956)

Table 1 Cont.

1958	Sierra Leone	Woodhead and Tweed (1960)
1960	Senegal, Ivory Coast	Woodhead and Tweed (1958)
1984	Wadides Sbeitlaffussen (Tunisia)	Wacorik (1984)
1986	Gambia	Foged (1986)
1987	Niger	Gasse (1987)

Table 2. Mean physico-chemical factors and *E. paludosa* abundances recorded along Kowie system in September 2012 to May 2013 [(TDS) total dissolved solids, (Rest) resistivity, (Temp) temperature, (Amm) ammonia, (Phos) phosphate].

Parameter	Sites						
	F1	F2	F3	F4	E1	E2	E3
TDS (ppt)	1.1±0.7	1.2±0.6	1.4±0.3	2.7±2.9	2.7±0.9	4.7±2.9	19±4.4
Cond (µS.cm ⁻¹)	0.9±1	1±0.7	2.4±0.8	2.6±0.4	5.2±1.6	9±4.3	34±4.4
Salinity (ppt)	0.8±0.5	0.9±0.4	1±0.2	1.1±0.1	2.1±0.8	3.7±2.5	17.2±4.7
pH	8±0.3	8.2±0.8	7.4±0.7	7.1±0.2	7.6±0.7	7.6±0.6	7.7±0.4
Temp (°C)	13.9±3.7	14.9±4.9	15.3±4.7	15.9±4.7	16.5±6.6	16.6±5.9	16.3±4.7
Depth (m)	0.5±0.1	1±0.1	0.8±0.2	0.5±0.2	2±0.5	2.8±0.5	4.3±0.8
Velocity (m.s ⁻¹)	0.1±0.1	0.3±0.3	0.3±0.3	0.3±0.2	0.2±0.1	0.2±0.1	0.1±0.2
Amm (mg.l ⁻¹)	0.1±0.1	0.1±0.1	0.2±0.2	0.1±0.1	0.3±0.2	0.3±0.4	0.5±0.3
Phos (mg.l ⁻¹)	0.8±1.3	0.5±0.3	2±4.5	0.5±0.7	0.2±0.3	0.2±0.1	1.8±2.9
Nitrate (mg.l ⁻¹)	1.3±2.1	13.8±18.7	1.5±2.1	0.9±2.3	1.9±4	8.1±10.3	2±3.4
Abund (no. l⁻¹)	75.5±75.7	581.3±429	2.8±449.2	4.3±543.5	6.5±536.8	2.8±534.9	16.3±89.2

CHOLNOKY (1966c) later questioned the validity of this variety, but as with ARCHIBALD (1983) he did not find enough intermediate cells in the populations he studied to justify combining the two taxa. This taxon is often sporadic in its occurrence, making it difficult to document the full range of morphological disparity within a population. In the present study we found a large population of *E. paludosa* and were able to document using LM (Figs 21–27) forms which would typically be thought to belong to *E. paludosa* var. *subsalina*, as well as those more typically considered as *E. paludosa* sensu stricto. This overlap of forms was also observed in Smith's mica mounted material (Figs 2–8), where the angle of the junction of the valve face and the keel changed with changes in cell size. Smith's original illustration showed a very sharp almost 90 degree angle, while Cleve illustrated a single smaller cell with a more gradually rounded less angled junction. Our population from the Kowie River had striae counts ranging from 21–26 in 10 µm and also displayed a range of morphological differences in the shape or angle of the junction of the valve face and the keel, all of which overlap with a similar range of

striae densities and shapes of the junction we observed in the type material (22–26 striae in 10 µm). For these reasons, we consider that *E. paludosa* var. *subsalina* is a possible synonym of the nominate variety; however, before this can be confirmed an examination of Cleve's type material would need to be undertaken as well as morphological and molecular studies of discrete populations.

Entomoneis paludosa distribution

In the current study, we found *E. paludosa* in abundances of 2.2–4.8% of the whole epipelon sample, which were high relative to the findings of ROS et al. (2009) from a tributary of the Segura River, south-east Spain [0–1.2% (epilithon) and 0–0.2% (epipelon)]. DEL-CRUZ et al. (2006) reported moderate to high abundances of *E. paludosa* at low nutrient levels in Australia but in the present study we observed a different scenario where *E. paludosa* densities were low at high nutrient concentrations. WECKSTRÖM & JUGGINS (2005) found *E. paludosa* had a maximum abundance of 1.1 individuals per litre in the Gulf of Finland, which is lower than that of the Kowie system.

Data-screening is a critical step in evidence-based status assessment, particularly for a species whose range is relatively unknown throughout the world (SENYATSO et al. 2013). Sampling bias and spatial scale issues resulting from uneven spatio-temporal sampling effort within and across countries might otherwise have compromised the analysis of occurrence records collated through search strategies of the type used in this study. In this study, there were relatively more records for southern and west Africa due to the levels of extensive research and experts available plus the geopolitical accessibility. Therefore, we acknowledge that this work is greatly intensified if different in-country experts are sought to validate records or provide distribution trends of the *E. paludosa* species, as it would reduce the effort required to complete assessments. Such assessments remain possible only if diatom experts are available in different countries.

At present, *E. paludosa* has been thought to occur only in Europe (Britain, Finland, France, Romania and Spain), America (Brazil, New Brunswick and United States of America) and Australasia (New Zealand and western Australia; SALVADOR in GUIRY & GUIRY 2014). Initial searches revealed no records in Africa because the species was previously identified using several synonyms such as *Entomoneis paludosa* (W. SMITH) REIMER 1975, *Amphicampa paludosa* (W. SMITH) RABENHORST 1864, *Amphiprora paludosa* W. SMITH 1853 and *Amphitropis paludosa* (EHRENBERG) RABENHORST 1868, which made it difficult to find any records (GUIRY 2013). All these misidentifications are ongoing, as is evident in the AlgaeBase website for the *E. paludosa* species.

Physico-chemical factors and *E. paludosa* communities

From the current study, the physico-chemical values were similar to those reported by GIFFEN (1970) for the Kowie system (South Africa). GIFFEN (1970) found that the species mainly occurred in water whose chloride concentrations varied from 0.42 to 16.67 $\mu\text{S}\cdot\text{cm}^{-1}$, which falls within our observed range of 0.9 to 34 $\mu\text{S}\cdot\text{cm}^{-1}$. In North western Great Plains (Montana, North Dakota, South Dakota, Wyoming) where *E. paludosa* is dominant, the species occurred in water having conductivity and pH of 3.28 $\mu\text{S}\cdot\text{cm}^{-1}$ and 8.6, respectively (PATRICK & REIMER 1975). Comparing with the Great Plains study, both conductivity and pH fell within the observed Kowie range, suggesting that species requires these conditions for it to grow.

The resistivity and pH levels were significantly correlated with *E. paludosa* abundances and played an important role in structuring the communities in this study. The relationship between diatoms and pH is strong, as pH exerts a direct physiological stress on diatoms and influences other water chemistry variables

such as resistivity and conductivity (BERE & TUNDISI 2011). Changes in the pH and resistivity levels might have had a physiological effect on the species, thereby affecting their abundances across the study months. Using RDA analysis, we observed that the *E. paludosa* populations were associated with nitrates and salinity (Fig. 49). These findings are similar to a recent study by DALU et al. (2014) on diatom communities showing that salinity was associated mostly with the estuary sites while nitrate were associated with mainly the polluted sites.

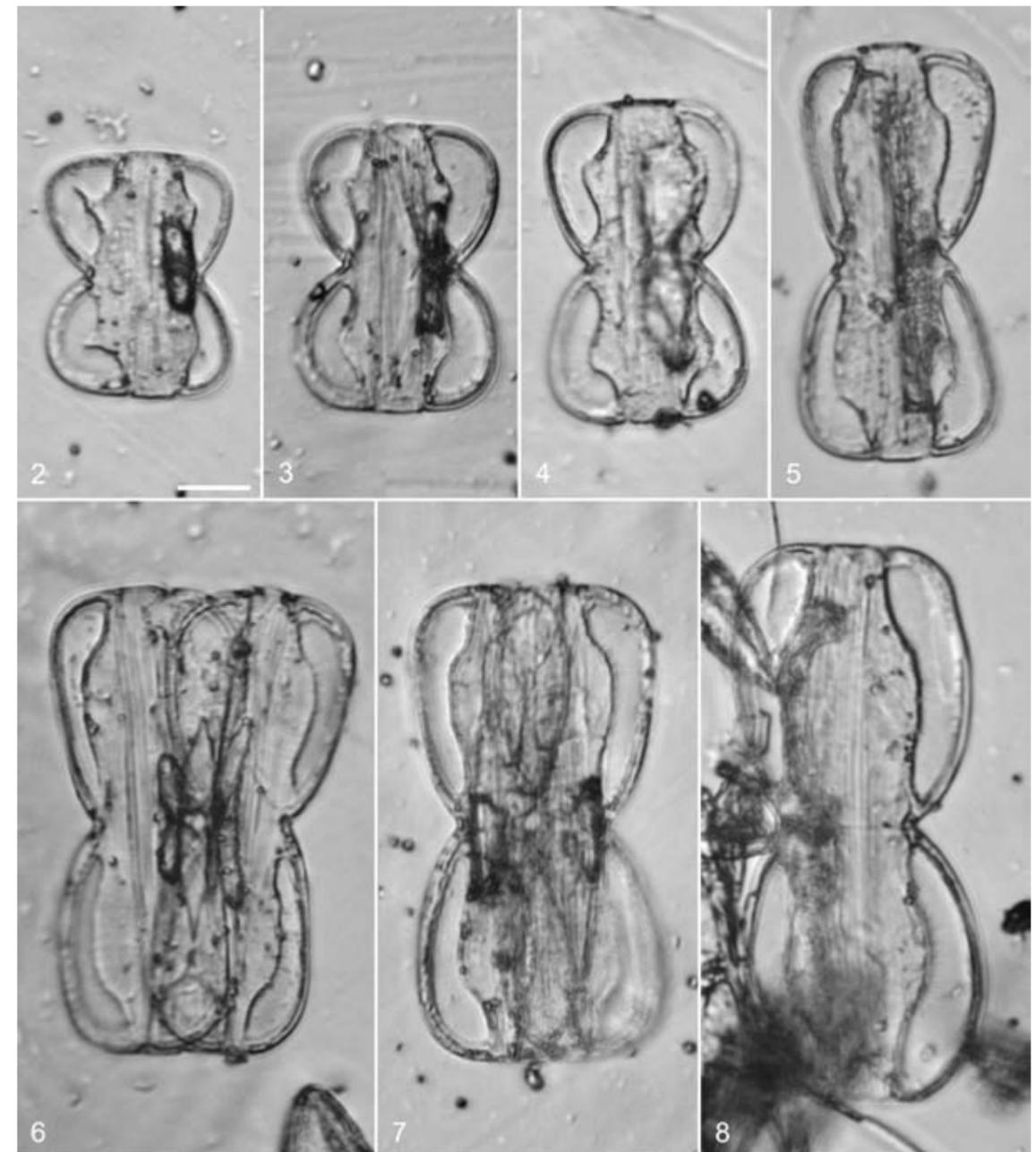
The observed sizes of *E. paludosa* specimens suggest that the species in South Africa is smaller compared to the species in other parts of the world (BAHLS 2012). In the Kowie River, the specimens were found in benthic samples and were highly motile and solitary. *Entomoneis paludosa* was found associated with *Cymbella* sp., *Cyclotella* sp., *Thalassiosira* sp., *Diatoma* sp., *Fragilaria* sp., *Gomphonema* sp., *Navicula* sp., *Nitzschia* sp., *Tryblionella* sp. and *Surirella* sp. (GIFFEN 1970; PATRICK & REIMER 1975; DALU et al. 2014). During the present study *E. paludosa* was associated with diatoms of the genera *Achnanthisdium* sp., *Cymbella* sp., *Cyclotella* sp., *Cymbella* sp., *Fallacia* sp., *Fragilaria* sp., *Gomphonema* sp., *Navicula* sp., *Nitzschia* sp., *Stauraphora* sp., *Pleurosigma* sp. and *Surirella* sp. similar to studies by GIFFEN (1970) and PATRICK & REIMER (1975).

ACKNOWLEDGEMENTS

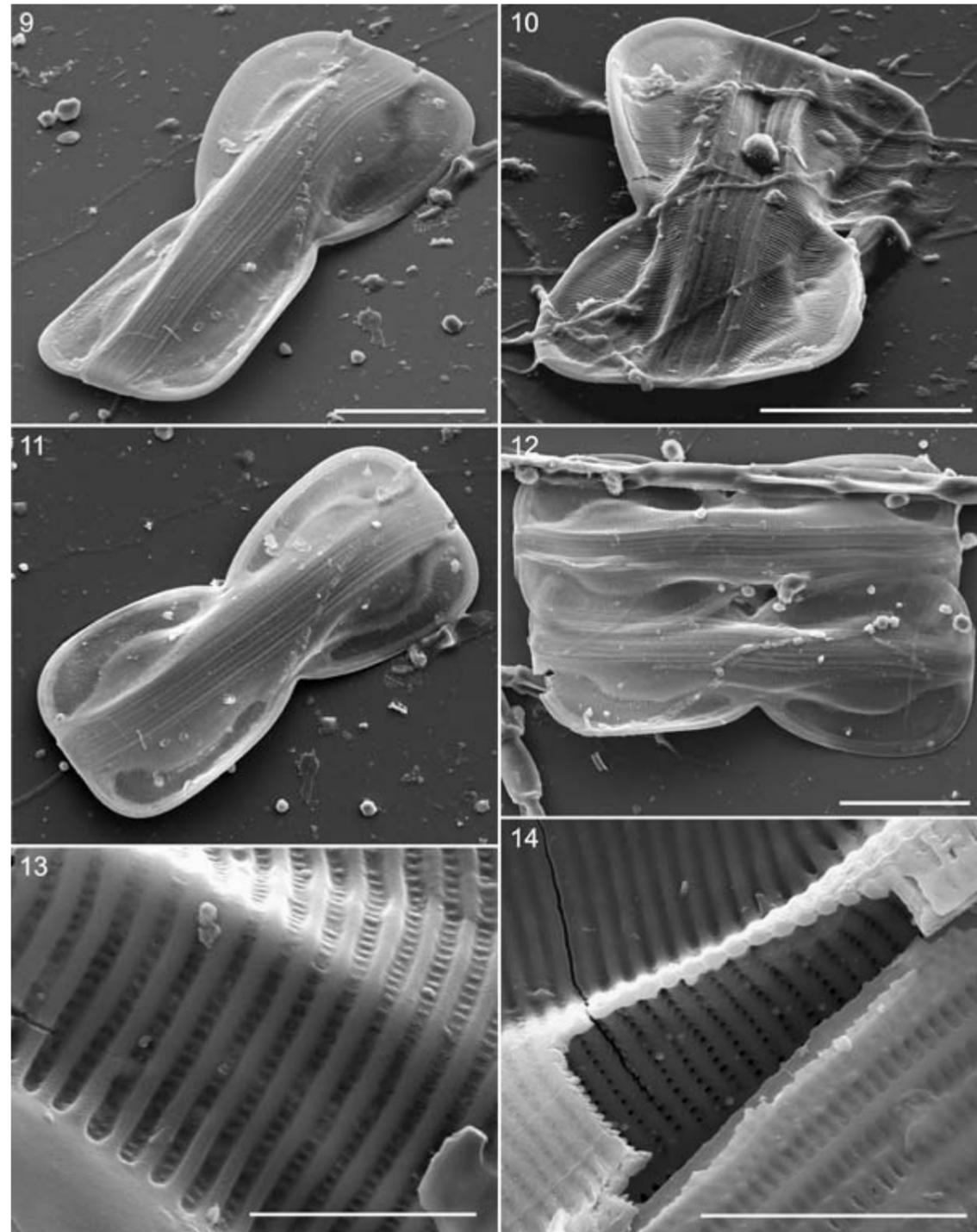
This work was supported by the Water Research Commission of South Africa, the National Research Foundation of South Africa and the Sandisa Imbewu initiative at Rhodes University. We thank Bart van de Vijver who located and provided access to the William Smith material at the Botanic Garden Meise. We are grateful to the North-West University and Rhodes University Electron Microscopy Units for access to equipment, and Lenin D Chari, Sydney Moyo and Likho Sikutshwa for assistance with the field work. Jonathan C Taylor is the recipient of South African National Research Foundation (NRF) incentive funding. Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and therefore the NRF does not accept any liability in regard thereto.

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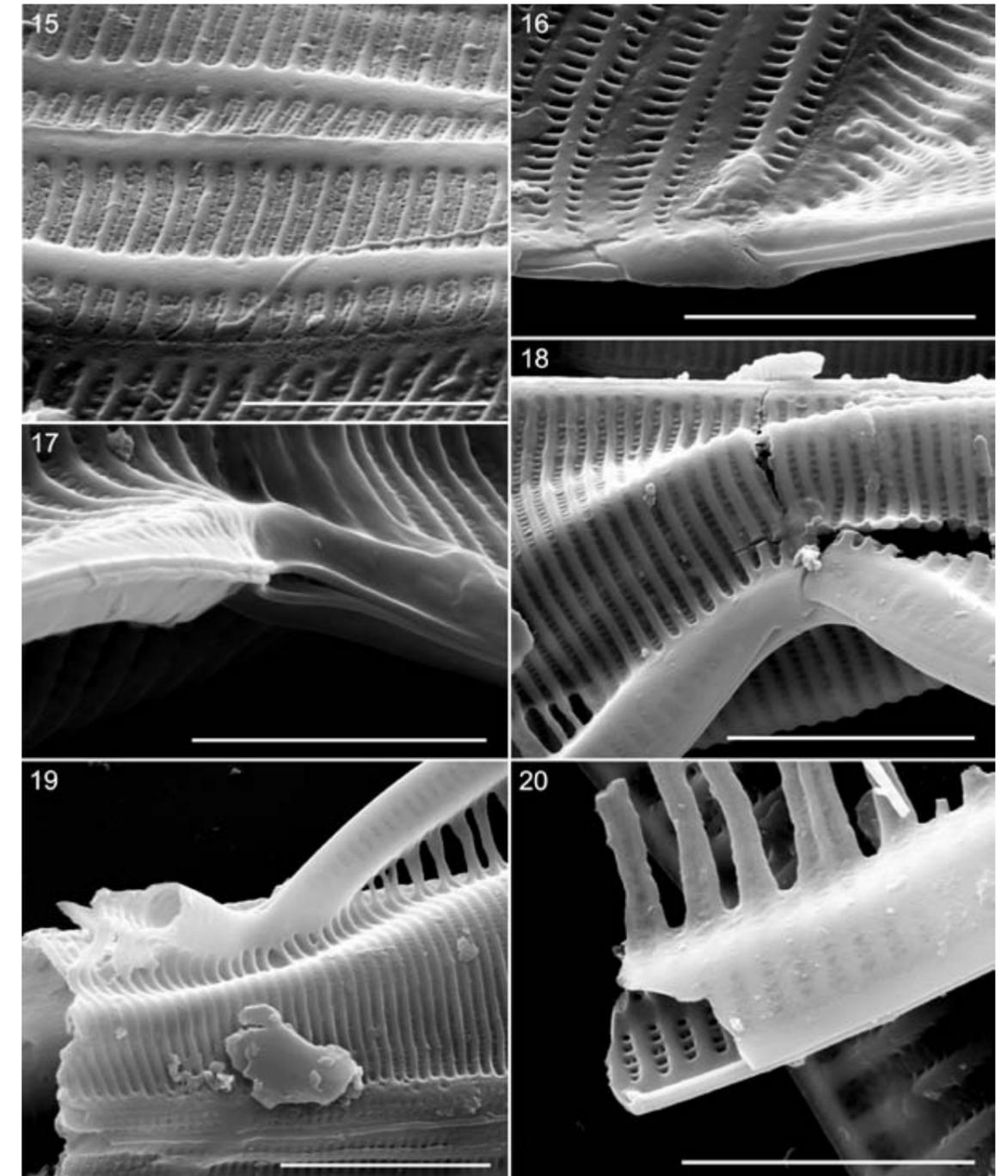
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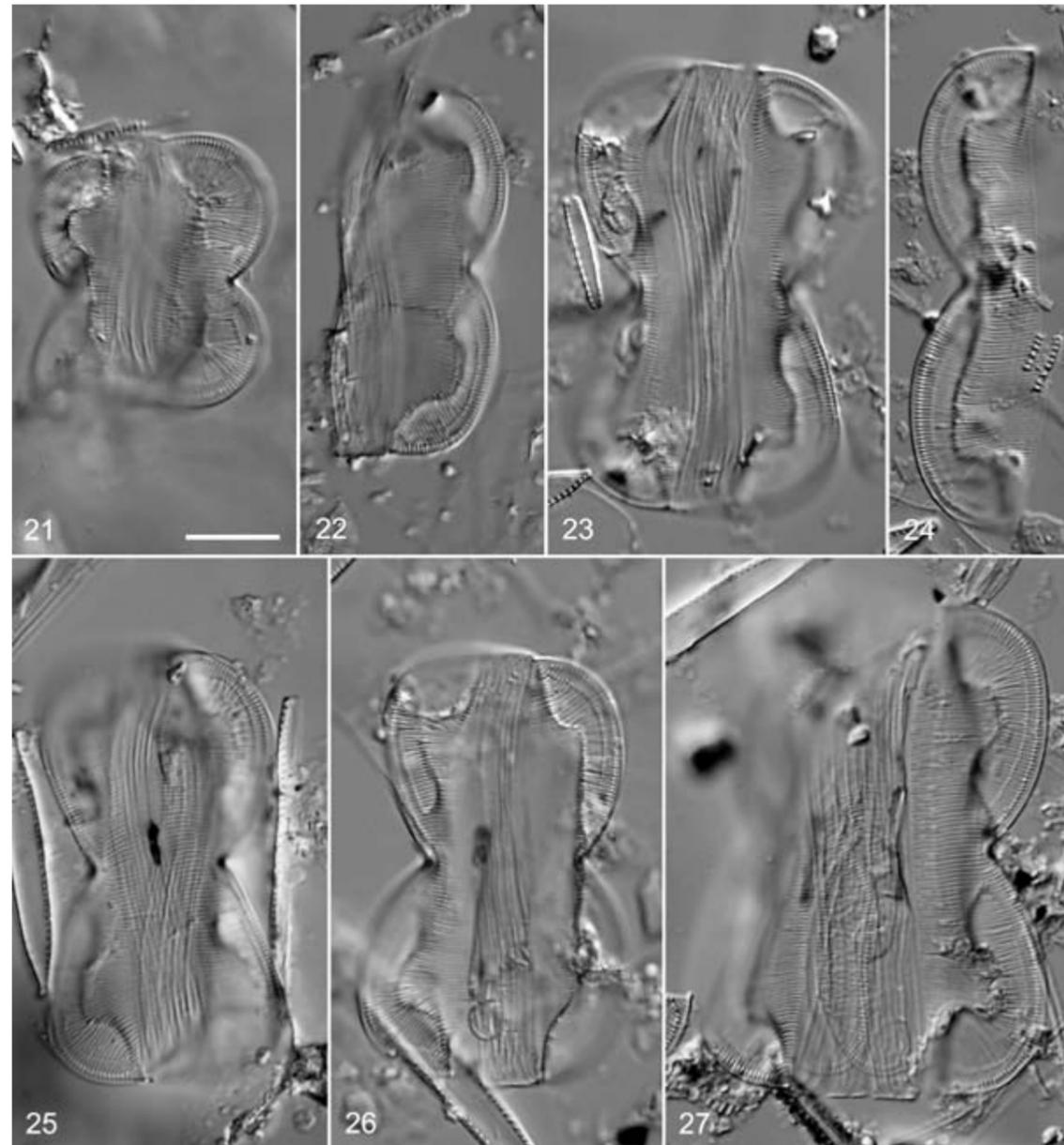
Figs 2–8. LM showing material on Smith's original mica preparation, taken from a packet marked 'Near Lewes, September 1953 in his own hand. Image series demonstrates changes in the shape of the junction between the keel and the valve face as overall cell size changes. Material is uncleaned and mounted in air. Scale bar 10 μm .



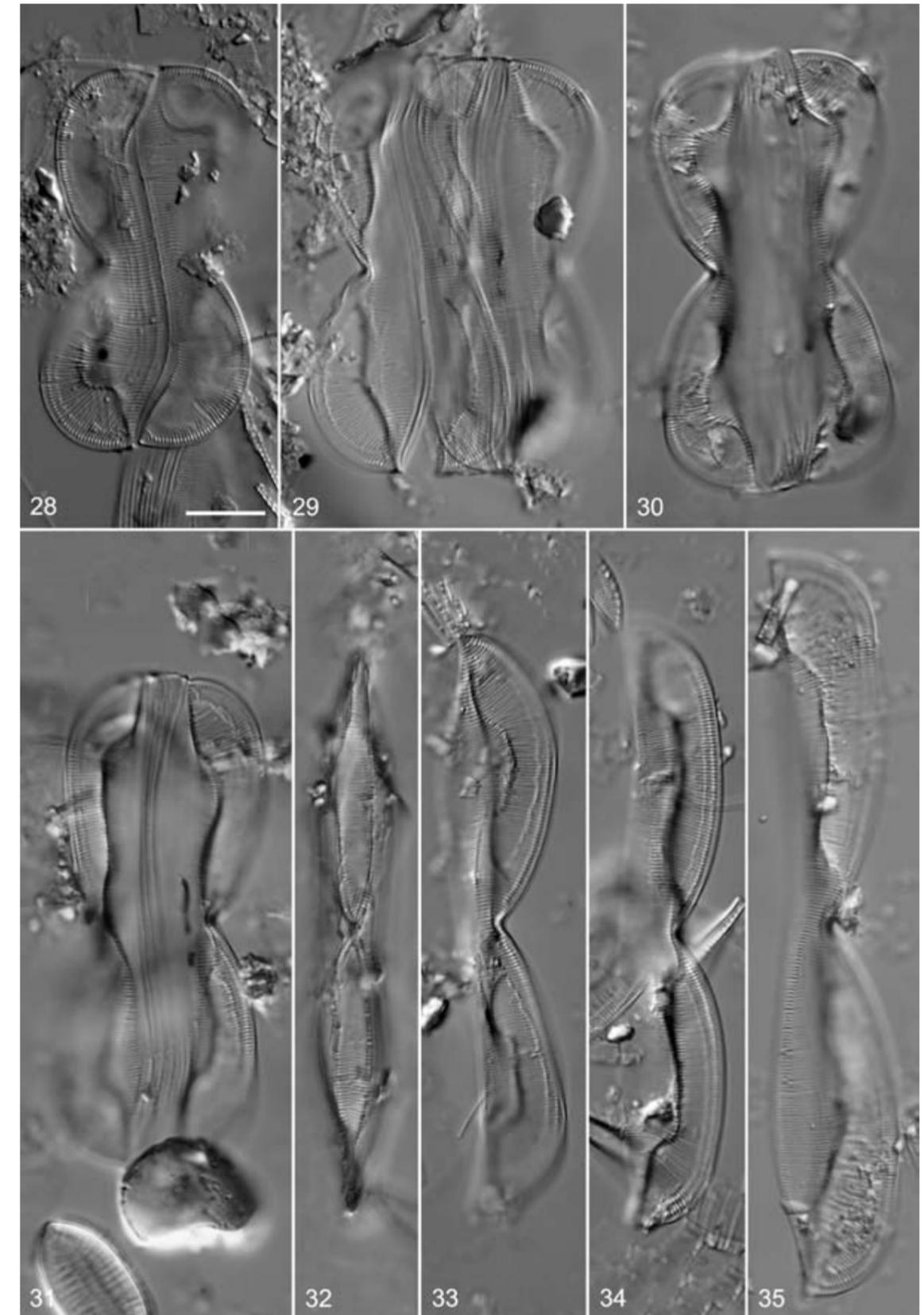
Figs 9–14. SEM showing material on Smith's original mica preparation: (9–12) View of complete valves; (13) Valve exterior near to mantle showing external occlusion of the areolae; (14) Broken section of the valve showing interior unoccluded areolae. Scale bars 20 μm (9–12), 2 μm (13) and 5 μm (14).



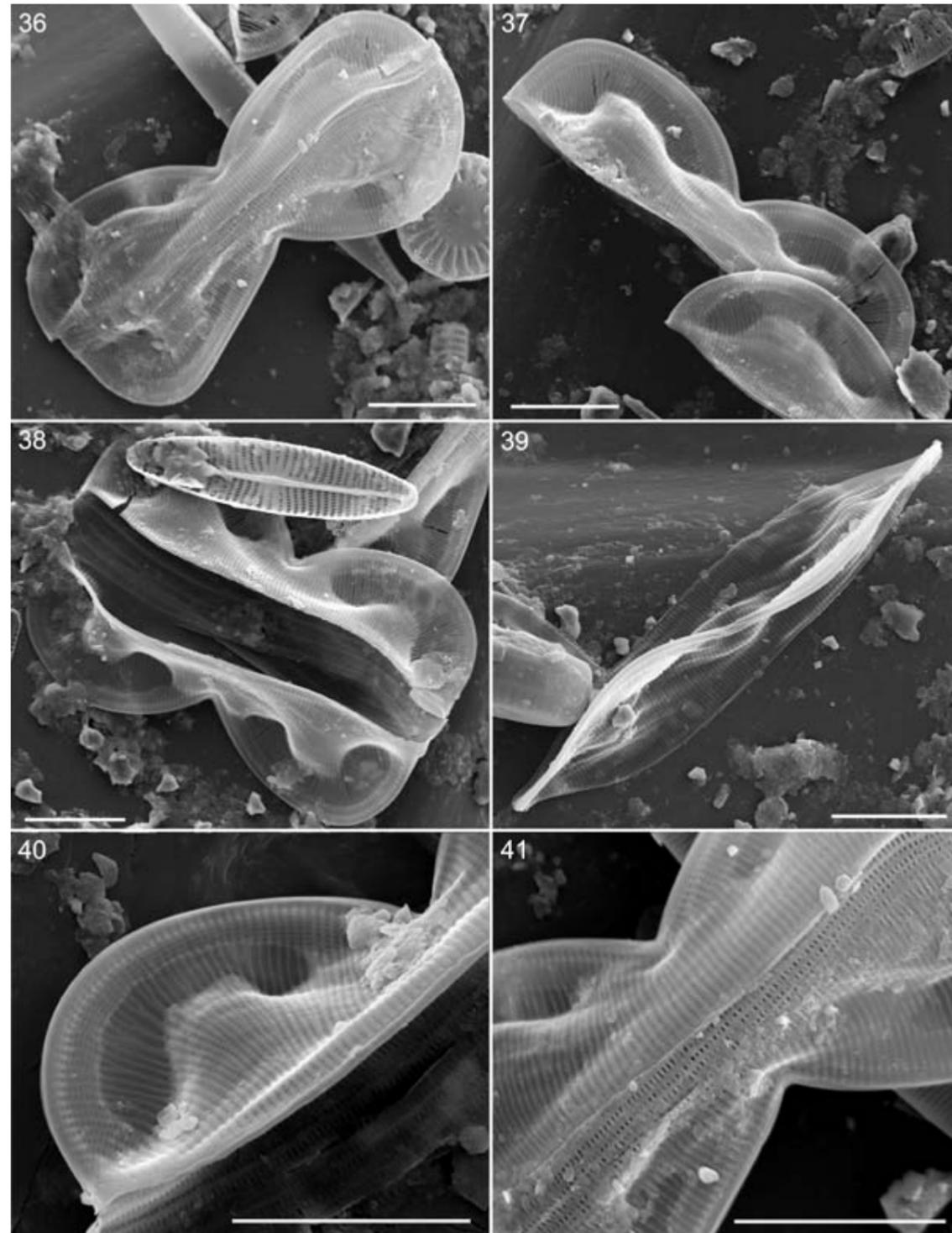
Figs 15–20. SEM showing material on Smith's original mica preparation: (15) A section of the girdle with larger areolae (externally occluded); (16) Terminal raphe ending, slightly bent; (17–19) Raphe and detail of central raphe ending; (20) Broken raphe showing small externally occluded areolae on the tubular raphe canal. Scale bar 2 μm (15), 5 μm (16 and 19), 3 μm (17 and 20), 4 μm (18).



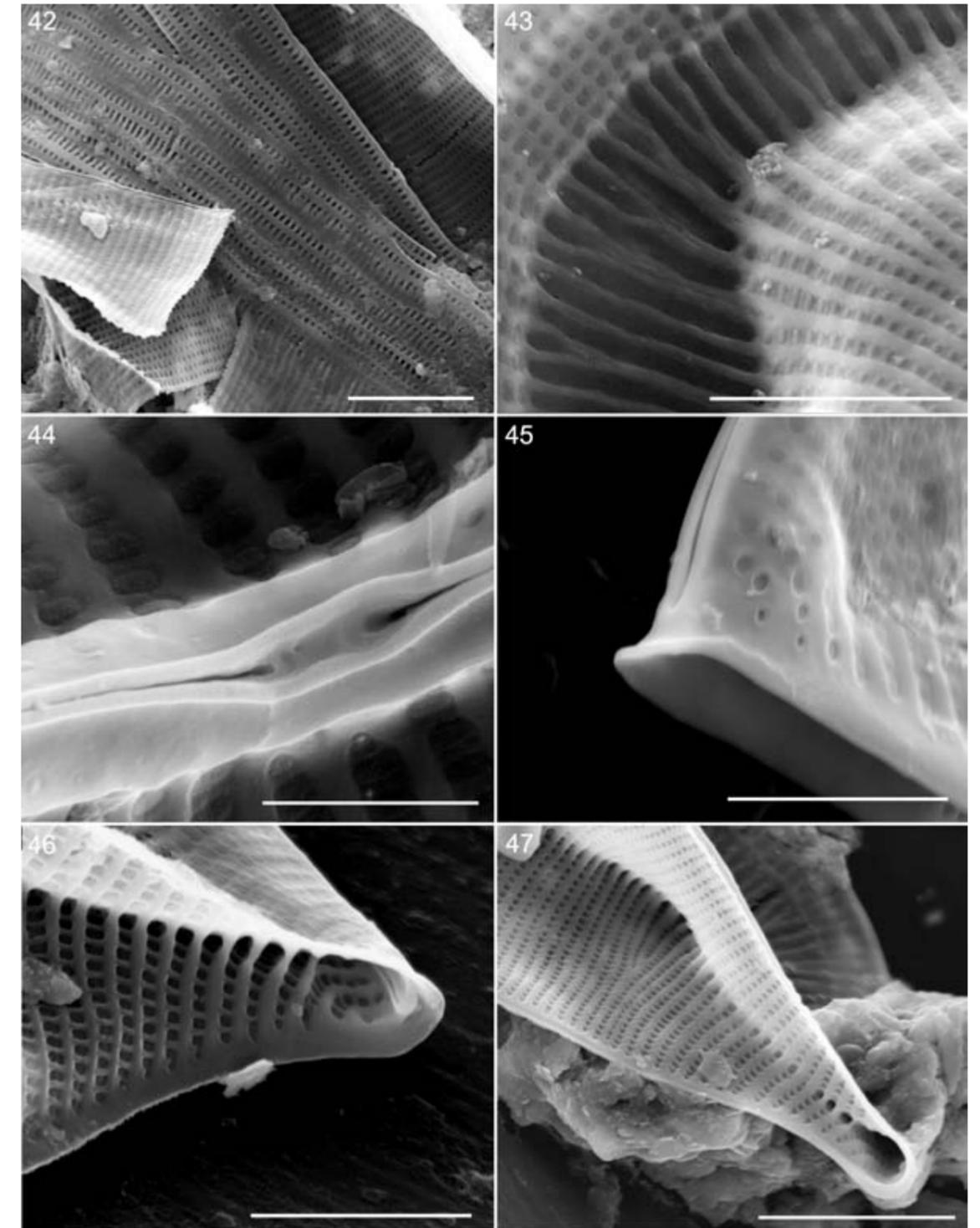
Figs 21–27. LM of cleaned material from the Kowie River, South Africa. Note the range of shapes and angles of the junction of the valve face and keel. Scale bar 10 μ m.



Figs 28–35. LM of cleaned material from the Kowie River, South Africa. Scale bar 10 μ m.



Figs 36–41. SEM of material from the Kowie River, South Africa: (36–39) Complete valves; (40) Junction of the valve face and keel; (41) Girdle region, detail of copulae. Scale bars 10 μm .



Figs 42–47. SEM of material from the Kowie River, South Africa: (42) Copulae perforated by elongated areolae; (43) Junction of the valve face and keel, showing externally occluded areolae; (44) Central raphe endings; (45) Terminal raphe ending; (46–47) Internal views showing the helctoglossa and the structure of the fibulae. Scale bar 5 μm (42 and 47, 44), 1 μm (45), 2 μm (46), 3 μm .

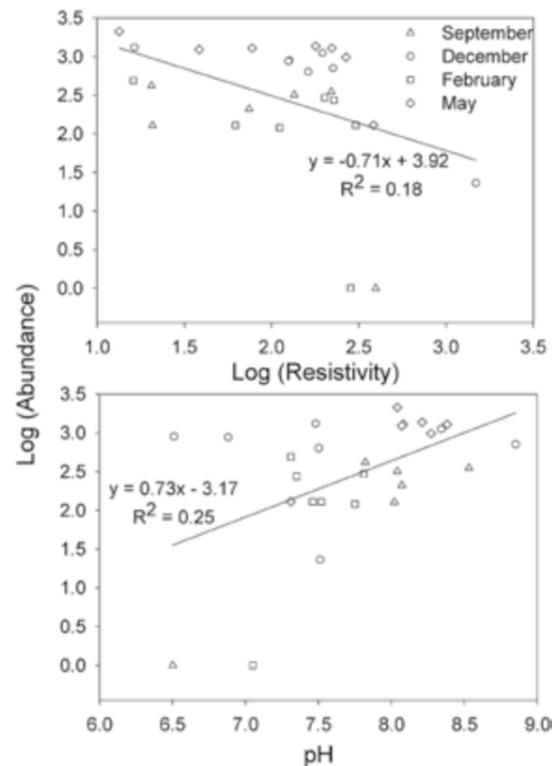


Fig. 48. Log (x+1) correlation of *E. paludosa* abundances with physico-chemical factors

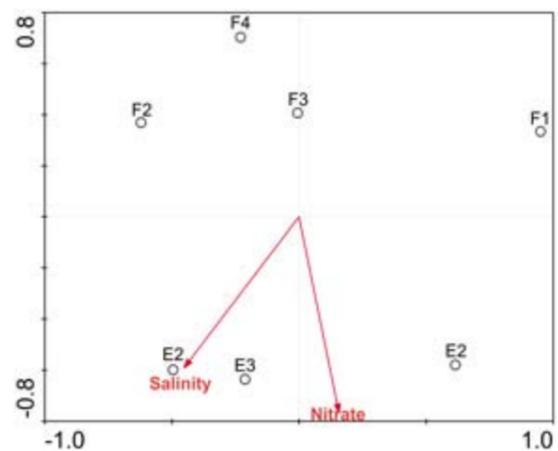


Fig. 49. Redundancy analysis plots showing effects of physico-chemical factors on *E. paludosa* taxa samples/sites in the ordination space of the first and second axes. [(F1–F5), (E1–E3) are the study sites where the species were collected].

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Received July 14, 2014

Accepted November 14, 2014