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Karenia cristata sp. nov. and *Karenia bicuneiformis* sp. nov. (Gymnodiniales, Dinophyceae): two new *Karenia* species from the South African coast

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In 1988 and 1989, an undescribed gymnodinioid dinoflagellate species turned the waters of the largest bay in South Africa, False Bay, to a dirty olive-green colour. The bloom was accompanied by extensive abalone (*Haliotis midae*) mortalities and noxious gases causing eye, nose, skin and throat irritations in humans. In 1995, another undescribed gymnodinioid species bloomed in the same bay but with no adverse effects on marine fauna or humans. These two species form an established component of the phytoplankton assemblage on the south coast of South Africa. They are described here as *Karenia cristata* Botes, Sym & Pitcher and *K. bicuneiformis* Botes, Sym & Pitcher. *Karenia cristata* has a straight apical groove elevated into an apical crest and extending down immediately to the right of the sulcal extension on the ventral side. The hypocone is asymmetrical, with the right lobe larger and more rounded than the left; the nucleus is central, with the bulk situated in the hypocone. Its pigment content is similar to that of *K. mikimotoi* and *K. brevis*. Other than *K. brevis*, *K. bicuneiformis* is significantly larger than the other *Karenia* species and is distinctly dorso-ventrally flattened. The hypocone is w-shaped and the epicone is conical, giving the cell a distinctly angular outline. Pairwise distance comparisons of partial large subunit (28S) rDNA sequences indicate that these two species are clearly different from other species within the genus.

INTRODUCTION

Until recently, considerable confusion existed worldwide with regard to the identity of many closely related species resembling *Gymnodinium mikimotoi* Miyake & Komani *ex* Oda *sensu lato* (Steidinger *et al.* 1998). Recent publications (Chang 1999; Daubjerg *et al.* 2000; Hansen *et al.* 2000; Yang *et al.* 2000, 2001) have partially clarified this confusion by revising the genus *Gymnodinium sensu lato* F. Stein (which resulted in the genus being split into four genera: *Gymnodinium sensu stricto* F. Stein *emend* Gert Hansen & Moestrup, *Karenia* Gert Hansen & Moestrup, *Karlodinium* J. Larsen and *Akashiwo* Gert Hansen & Moestrup) and by describing species such as *Karenia brevisulcata* (Chang) Gert Hansen & Moestrup, *K. digitata* Yang, Takayama, Matuoka & Hodgkiss and *K. longicanalis* Yang, Hodgkiss & Gert Hansen.

As part of a larger study, several gymnodinioid species present on the south coast of South Africa were studied by light microscopy (LM), scanning electron microscopy (SEM) and with respect to pigment content and partial large subunit ribosomal DNA (LSU rDNA, 28S rDNA) sequences. During the course of this study (the results of which will be published separately), it became evident that two of these species are markedly different from their counterparts and should therefore be described as new species, here named as *K. cristata* Botes, Sym & Pitcher, *sp. nov.* and *K. bicuneiformis* Botes, Sym & Pitcher, *sp. nov.* The former is known in the literature as *Gymnodinium* sp. (Horstman *et al.* 1991; Matthews & Pitcher 1996; Pitcher & Matthews 1996) or *Gymnodinium* cf. *mikimotoi* (Pitcher & Calder 2000); it bloomed at the H.F. Verwoerd Nature Reserve, South Africa (Fig. 1) during April 1988 and again during March 1989 (Horstman *et al.* 1991). It subsequently bloomed again at Gordon's Bay in 1995–1996 (Pitcher & Matthews 1996) and was responsible for eye, nose, throat and skin irritations in humans as well as extensive mortalities of sub- and intertidal fauna. Blooms of *K. cristata* were associated with calm periods and sea surface temperatures exceeding 17°C, followed by an onshore wind necessary to concentrate the blooms inshore (Horstman *et al.* 1991). In 1995, *K. bicuneiformis* bloomed in Gordon's Bay, South Africa (Fig. 1), and again in 1997. No adverse effects were attributed to this species. Both species form an established component of the phytoplankton on the south coast of South Africa and are typically present in False Bay and Walker Bay. The type localities of *K. cristata* and *K. bicuneiformis* are H.F. Verwoerd Nature reserve and Gordon's Bay, respectively (Fig. 1).

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MATERIAL AND METHODS

Samples and sampling

Water samples were collected at Gordon's Bay (Fig. 1) and preserved with 5% formaldehyde (buffered with $CaCO_3$, AR grade, pH $\geq$ 7). For live observation, pigment extraction and LM, samples were collected with a 20 μm net and concentrated by means of a 10 μm mesh.

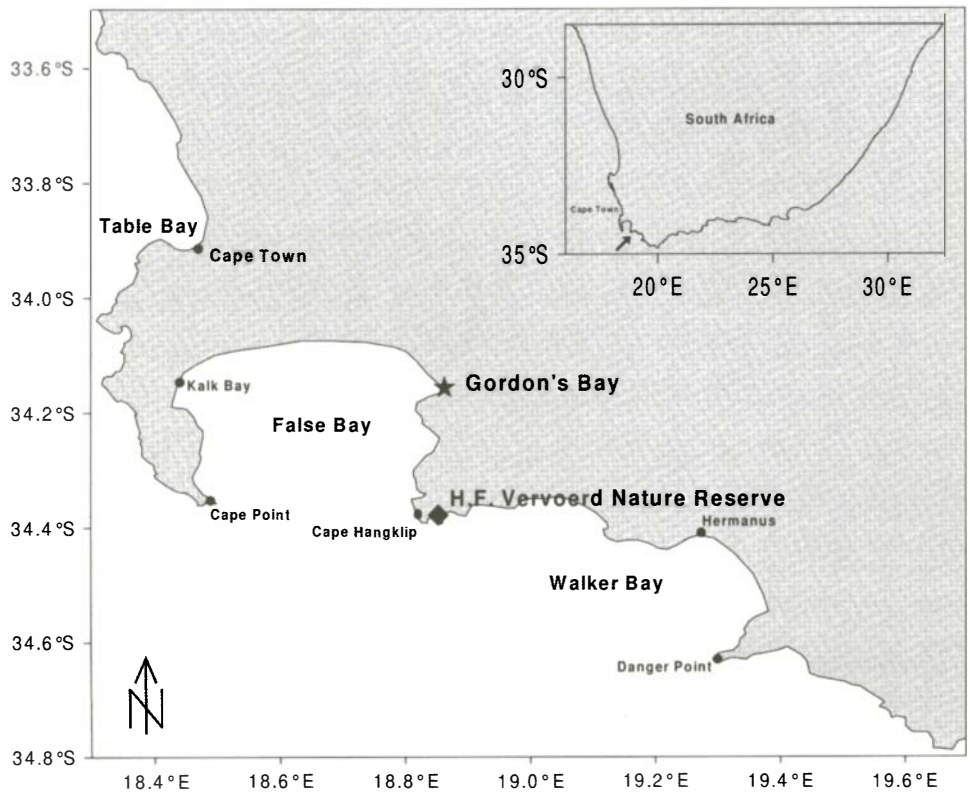


Fig. 1. Sites within False Bay and Walker Bay, South Africa, where *K. cristata* and *K. bicuneiformis* have been recorded. The type locality of *K. cristata* (♦) and *K. bicuneiformis* (★) are indicated by the respective symbols.

Light microscopy

Specimens of both species were observed, measured and photographed with either an Olympus B201 light microscope equipped with an Olympus DP10 digital camera or a Zeiss Axiophot photomicroscope with a photographic set-up that included the production of videoprints, video recording and micrographs.

SEM

Scanning electron micrographs were obtained according to the technique developed by Botes *et al.* (2002), except that cells of *K. cristata* and *K. bicuneiformis* were preserved with buffered 5% formaldehyde, instead of osmium tetroxide (OsO_4).

Pigment composition

Cells of *K. cristata* were filtered through 4.7 cm GF/F (Whatman International, Maidstone, UK) microfibre filter paper. Pigments were extracted in 2 ml of 90% acetone using ultrasonication and centrifugation and were analysed by high-performance liquid chromatography (HPLC) according to Barlow *et al.* (1997) at a flow rate of 1 ml min^{-1} on a C-8 column using a binary linear gradient. Solvent A consisted of 70:30 (v/v) methanol:1 M ammonium acetate and solvent B was 100% methanol. Pigments were detected by absorbance at 440 nm using a UV 6000 diode array detector (Thermo Separations Products, San Jose, CA, USA). Pigments were identified by retention time and appropriate diode array spectra (Jeffrey & Vesk 1997). Chlorophyll *a* and *b,* and α- and β-carotene standards were obtained from Sigma Chemical Company (Vorna Valley, South Africa) and the Water Quality Institute (VKI), Hørsholm, Denmark.

Table 1. Oligonucleotide primer sequences used to amplify the partial LSU (28S) rDNA sequences.

Primer name	Primer sequence (5′–3′)
D1R	5′-ACCCGCTGAATTTAAGCATA-3′
D3Ca	5′-ACGAACGATTTGCACGTCAG-3′

DNA extraction, amplifications and determination of partial LSU (28S) rDNA sequences (D1–D3)

For *K. cristata,* isolation and preparation of individual live cells and polymerase chain reaction (PCR) protocols of Bolch (2001) were followed to amplify ~ 850 base pairs of LSU rDNA, using terminal primers D1R (Scholin *et al.* 1994) and D3Ca (Lenaers *et al.* 1989) (Table 1). The protocols for PCR amplification and thermal cycling of Hansen *et al.* (2000) were followed but with a reaction volume of 25 µl, 3 mM $MgCl_2$ and Bioline *Taq* polymerase (Whitehead Scientific, Cape Town, South Africa). DNA fragments were checked on 1% agarose gels containing ethidium bromide, cut with a sterile surgical blade and purified using the QIAquick Gel Extraction Kit (Qiagen, Southern Cross Biotechnology, Cape Town, South Africa). Both strands of PCR product were sequenced by using the ABI PRISM BigDye terminator Cycle-Sequencing Ready Reaction Kit (v. 2) (PE Biosystems, Johannesburg, South Africa). The sequence reactions were run

Table 2. Specimens included in pairwise distance comparisons. Two *Akashiwo sanguinea* isolates and two *Karenia mikimotoi* isolates were used to calculate pairwise distances for isolates within species, and the various *Karenia* species were used to calculate pairwise distances for species within the same genus.

Species name (names in parentheses are as they appear in GenBank)	Origin	Isolated by	Accession number (GenBank)
A. sanguinea	False Bay, South Africa	L. Botes	AY518424
A. sanguinea (*Gymnodinium sanguineum*)	Marlborough Sounds, New Zealand	L. Rhodes	U92253
Karenia brevis	Florida, USA	W.B. Wilson	AF200677
K. brevisulcata	Wellington Harbour, New Zealand	L. Mackenzie	AY243032
K. cristata	False Bay, South Africa	L. Botes	AY525907
K. mikimotoi	Seto Island Sea, Japan	T. Ikeda & S. Mutsuno	AF200681
K. mikimotoi	Plymouth, UK	D. Harbor	AF200678
K. bicuneiformis (*Gymnodinium* sp.)	Hawke's Bay, New Zealand	A. Haywood	U92251
Karenia sp. (*Gymnodinium* sp.)	Chile	—	AF200677

on an ABI PRISM 3100 Genetic Analyser, following the recommendations of the manufacturer.

Pairwise distance comparisons

Pairwise distance comparisons between isolates of the same species, and between species of the same genus were calculated based on the partial LSU rDNA sequences (Table 2). Sequences used for parsimony comparisons were between 543 and 962 base pairs in length. The Tamura–Nei Model (Tamura & Nei 1993) was used to calculate the distances using PAUP*4.0b8 (Swofford 1998) (Table 4). Note that the pairwise distances for *K. bicuneiformis* were based on the New Zealand isolate (U92251).

OBSERVATIONS

Karenia cristata Botes, Sym & Pitcher, *sp. nov.*

Figs 2–9

Cellulae dorsiventraliter complanatae, *c.* 25.7 μm (24.05–27.35 μm, n = 15) longae, *c.* 24.29 μm (21.79–26.79 μm, n = 15) latae, *c.* 10 μm (n = 1) altae. Hypoconus asymmetricus excentricus lobo dextero maiore rotundatioreque quam lobo sinistro. Canalis apicalis rectus in cristam apicalem elevatus et ad juncturam cinguli cum sulco in superficie ventrali et secus distantiam tantum brevem in superficie dorsali extensus. Nucleus in hypocono centralis sed aliquantum in epicono extensus. Sulcus in epiconum extensus et in hypoconum versus antapicem dilatatus. Spatium inter extrema cingulo latitudine hujus duplo latius. Chloroplasti numerosi chlorini, respectu formam variabiles.

Cells dorso-ventrally flattened, *c.* 25.7 μm (24.05–27.35 μm, n = 15) long, *c.* 24.29 μm (21.79–26.79 μm, n = 15) wide, *c.* 10 μm (n = 1) deep. The hypocone is asymmetrical, with a larger and more rounded right lobe. The apical groove is straight and elevated into an apical crest, extending to the cingulum–sulcus junction on the ventral side of the cell and only a short distance on the dorsal side of the cell. The nucleus is central in the hypocone, but extends somewhat into the epicone. The sulcus extends onto the epicone and flares out to the antapex. The cingulum displaced twice its own width. Chloroplasts are numerous, yellow-green and variable in shape.

HOLOTYPE: Fig. 2.

ISOTYPE: SEM stub containing specimens of *K. cristata* (accession number J 100091) was submitted to the Moss Herbarium, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa.

ETYMOLOGY: Latin *cristata,* crest.

TYPE LOCALITY: H.F. Verwoerd Nature Reserve, South Africa (Fig.1).

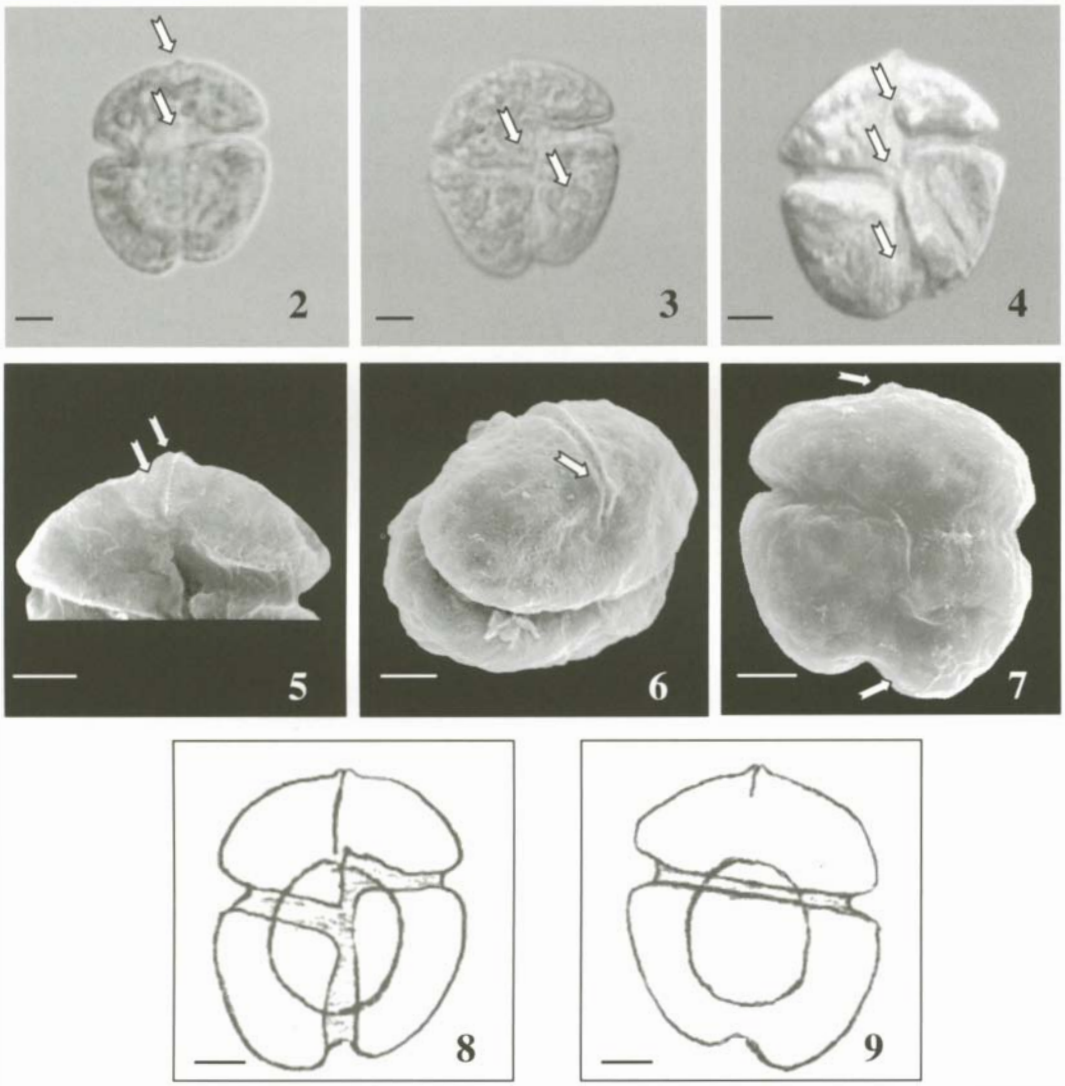
DISTRIBUTION: False Bay and Walker Bay, South Africa (Fig. 1).

Motile cells of *K. cristata* are dorso-ventrally flattened, *c.* 25.7 μm (24.05–27.35 μm, n = 15) long, *c.* 24.29 μm (21.79–26.79 μm, n = 15) wide and *c.* 10 μm deep (n = 1; Fig. 6). The hypocone is asymmetric with a larger and more rounded right lobe (Figs 2–4, 7). The cingulum is median to premedian (Fig. 3) with a displacement of twice its own width (Fig. 4). The sulcus extends onto the epicone and widens towards the antapex on the hypocone (Fig. 4). The apical groove is straight (Figs 2, 5, 6) and elevated to form a crest on the apex (Figs 2, 4, 5). On the ventral surface the apical groove starts immediately to the right of the sulcal extension (Fig. 5) and extends for a short distance onto the dorsal surface (Fig. 6). The nucleus is round to oval and situated in the centre of the cell, with the bulk of the nucleus situated in the hypocone (Fig. 2). Several yellow-green chloroplasts that are variable in shape are present (Fig. 3).

The swimming behaviour is similar to the 'falling leaf' motion of *K. mikimotoi* (Miyake & Komani *ex* Oda) Gert Hansen & Moestrup (Matsuoka *et al.* 1989), although it seems to be affected by the large right lobe. While rotating around its own axis during forward propulsion, the asymmetric nature of the hypocone makes it appear that the right lobe labours 'uphill' but falls 'downhill' effortlessly.

The main carotenoid of *K. cristata* is fucoxanthin; like *K. mikimotoi* and *K. brevis* (Davis) Gert Hansen & Moestrup, this species contains the accessory pigment gyroxanthin-diester (Table 3). Pairwise distance values obtained from comparison of partial LSU rDNA sequences confirm the status of *K. cristata* as a new species, with values falling within the range calculated for species within the same genus but not within the range calculated for isolates within the same species (Table 4).

Karenia cristata, like *K. brevis* and *K. brevisulcata* (Table 3), contains an aerosol toxin which causes coughing, burning of nasal passages, difficulty in breathing, stinging of the eyes and irritation to the skin. A sea urchin bioassay indicated that a cell concentration of $\sim 0.5 \times 10^6$ cells l^{-1} of *K. cristata* inhibits normal development of sea urchin embryos (Horstman *et al.* 1991). This species also has adverse effects on abalone (*Haliotis midae* Linnaeus) larvae (2 days old) and spat (3 mm animals) with a LC10 value of *c.* 1,143,184 (range 1,085,506–1,200,862) cells l^{-1} for abalone larvae and a LC10



Figs 2–9. *Karenia cristata* sp. nov. Scale bar = 5 μm. Live cells in LM (Figs 2–4); formalin-preserved cells in SEM (Figs 5–7); line drawings (Figs 8, 9).

Fig. 2. Typical cell shape in ventral view, showing the asymmetric hypocone, nucleus position (bottom arrow) and the apical crest with the apical groove (top arrow).

Fig. 3. Ventral view of the cell, showing the cingular displacement (top arrow) and the chloroplasts (bottom arrow) that are variable in shape.

Fig. 4. Ventral view showing the cingular displacement (middle arrow) and the sulcus that extends anteriorly onto the epicone (top arrow), and flares out posteriorly to the antapex (bottom arrow).

Fig. 5. Ventral view of the apical crest (bottom arrow) with the apical groove (top arrow).

Fig. 6. Anterior view of the apical groove (arrow) extending from the ventral side across to the dorsal side.

Fig. 7. Dorsal view of the apical crest (top arrow) and asymmetric hypocone (bottom arrow).

Figs 8, 9. Line drawings of the ventral and dorsal surfaces, respectively.

value of *c.* 2,526,651 (range 2,452,837–2,600,465) cells l^{-1} for abalone spat (Botes *et al.,* in press). Cell concentrations in excess of 10^6 cells l^{-1} were reported when 40 tons of adult abalone was washed ashore during the 1989 bloom (Horstman *et al.* 1991).

Karenia bicuneiformis Botes, Sym & Pitcher, *sp. nov.*

Figs 10–19

Cellulae dorsiventraliter complanatae, *c.* 36.25 μm (33.88–38.62 μm, n = 15) longae, *c.* 33.83 μm (31.3–36.36 μm, n = 15) latae, *c.* 5 μm (n = 1) altae. Hypoconus bicuneiformis, epiconus acute conicus, cellula igitur quinquangularis. Lobus uterque hypoconi fortasse indentatus. Canalis apicalis rectus, ad juncturam cinguli sulcique in superficie ventrali et secus tantum distantia brevi in superficie dorsali extensus. Nucleus ovalis in latere sinistro hypoconi situs. Extensio sulci in epicono praesens. Spatium inter extrema cingulo latitudinem hujus aequans. Chloroplasti numerosi chlorini respectu formam variabiles. Chloroplasti necati conservatique globosi.

Cells dorso-ventrally flattened, *c.* 36.25 μm (33.88–38.62 μm, n = 15) long, *c.* 33.83 μm (31.3–36.36 μm, n = 15) wide and *c.* 5 μm (n = 1) deep. The hypocone is w-shaped and the epicone is sharply conical, giving the cell a markedly angular outline. Each lobe of the hypocone can be indented at times. The apical groove is straight, extending down to the cingulum–sulcus junction on the ventral side of the cell and only a short distance on the dorsal side of the cell. The nucleus is oval and on the left side of the hypocone. An ex-

Table 3. Comparison of features for differentiating related *Karenia* species.

Parameters	*K. cristata* sp. nov.	*K. bicuneiformis* sp. nov.	*K. mikimotoi* Takayama & Adachi (1984)	*K. brevis* Takayama (1990)	*K. brevisulcata* Chang (1999)	*K. longicanalis* Yang *et al.* (2001)	*K. digitata* Yang *et al.* (2000)
Cell length (μm)	25.70 (24.05–27.35, n = 15)	36.25 (33.88–38.62, n = 15)	25–35	18–40	19–25 (big), 13–17 (small)	25.9 (20.4–31.4, n = 50)	21.46 (18.5–24.42, n = 50)
Cell width (μm)	24.29 (21.79–26.79, n = 15)	33.83 (31.3–36.36, n = 15)	23–33	15–70	18–22 (big), 10–17 (small)	21.1 (16.8–25.4, n = 50)	18.25 (15.71–20.79, n = 50)
Dorso-ventrally flattened	*c.* 10 μm in side view	*c.* 5 μm in side view	no data	no data	no data	no data	no data
Hypocone, shape	aysmmetric	W-shaped	bilobed	bilobed	circular outline	hemispherical	rounded to hemispherical
Sulcus extension	present	present	present	present	present	absent	present
Apical groove, ventral	long	long	long	long	short	long	long
Apical groove, dorsal	short	short	short	short	short	long	short
Apical crest	present	absent	absent	absent	absent	absent	absent
Carina	absent	absent	absent	present	absent	absent	absent
Nucleus	round to oval, central	oval, left in hypocone	reniform, left in hypocone	round, left in hypocone	round to horizontally elongated, left to central in hypocone	round, central	round, subcentral
Chloroplasts	variable in shape	variable, disc-shaped upon fixation	shapeless	peripheral	elongated	round	shapeless
Aerosol toxin	present	absent	absent	present	present	absent	absent
Pigment content	chlorophyll c_2, c_3 and a; butanoyloxy-fucoxanthin; fucoxanthin; hexanoyloxy-fucoxanthin; diadinoxanthin; gyroxanthin-diester; β-carotene	no data	chlorophyll c_1/c_2, c_3 and a; butanoyloxy-fucoxanthin; fucoxanthin; hexanoyloxy-fucoxanthin; diadinoxanthin; gyroxanthin-diester; β-carotene[1]	chlorophyll c_1/c_2, c_3 and a; butanoyloxy-fucoxanthin; fucoxanthin; hexanoyloxy-fucoxanthin; diadinoxanthin; gyroxanthin-diester; β-carotene[2]	no data	no data	no data

[1] Hansen *et al.* (2000).
[2] Millie *et al.* (1995).

Table 4. Pairwise distance values, obtained from comparison of LSU rDNA sequences, calculated for species within the same genus and isolates within the same species. Note that the pairwise differences for *K. bicuneiformis* were based on the sequence of *Karenia* sp. (*Gymnodinium* sp. U92251).

Pairwise distance comparison (Taimura–Nei model)		Actual value of comparison between individuals	Mean	Range
Isolates within the same species (examples)			3.72004×10^{-4}	0.00000000–0.0008178
	K. mikimotoi (UK)–*K. mikimotoi* (Japan)	0.00012345	—	—
	A. sanguinea (Denmark)–*A. sanguinea* (New Zealand)	0.00064774	—	—
Species within the same genus (examples)			0.024450356	0.01335893–0.03854208
	K. cristata (South Africa)–*K. brevisulcata* (New Zealand)	0.01426310	—	—
	K. cristata (South Africa)–*Karenia* sp. (Chile)	0.01189127	—	—
	K. cristata (South Africa)–*K. mikimotoi* (Japan)	0.02132520	—	—
	K. cristata (South Africa)–*K. brevis* (USA)	0.01927742	—	—
	K. brevisulcata (New Zealand)–*Karenia* sp. (Chile)	0.01389328	—	—
	K. mikimotoi (Japan)–*K. brevis* (USA)	0.01335893	—	—
	K. bicuneiformis (New Zealand)–*K. cristata* (South Africa)	0.02615790	—	—
	K. bicuneiformis (New Zealand)–*K. brevisulcata* (New Zealand)	0.03757461	—	—
	K. bicuneiformis (New Zealand)–*K. mikimotoi* (Japan)	0.03854208	—	—
	K. bicuneiformis (New Zealand)–*K. brevis* (USA)	0.03474694	—	—

tension to the sulcus is present on the epicone. The cingulum is displaced by one cingulum width. Chloroplasts are numerous, yellow-green and variable in shape. Preserved chloroplasts become circular.

HOLOTYPE: Fig. 14.

ISOTYPE: SEM stub containing specimens of *K. bicuneiformis* (accession number J 100090) was submitted to the Moss Herbarium, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa.

ETYMOLOGY: Latin *bi,* two and *cuneiformis,* wedge shaped.

TYPE LOCALITY: Gordon's Bay, South Africa (Fig. 1).

DISTRIBUTION: False Bay and Walker Bay, South Africa (Fig. 1).

Motile cells of *K. bicuneiformis* are dorso-ventrally flattened, *c.* 36.25 μm (33.88–38.62 μm, $n = 15$) long, *c.* 33.83 μm (31.3–36.36 μm, $n = 15$) wide and *c.* 5 μm deep ($n = 1$; Fig. 16). The hypocone is w-shaped and the epicone is conical, giving the cell a markedly angular outline (Figs 10, 14). The hypocone may occasionally possess indentations on its dorsal and ventral surfaces (Figs 13, 17). The cingulum is median to premedian and is displaced by one cingulum-width (Fig. 14). The sulcus extends onto the epicone and does not flare in the hypocone. The apical groove is straight (Figs 10, 17). On the ventral side, the apical groove starts immediately to the right of the sulcal extension (Fig. 14) and extends for a short distance onto the dorsal side (Fig. 15). The nucleus is oval and situated on the left side of the hypocone (Fig. 11). Several variably shaped, yellow-green chloroplasts are present (Fig. 12) and, on fixation, form round discs (Fig. 11).

The swimming behaviour of *K. bicuneiformis* is smooth and slow and similar to the 'falling leaf' motion described by Matsuoka *et al.* (1989).

Pigment data are unavailable for this species; however, based on its affiliation to the genus *Karenia* and the colour of the species when observed with LM, this species most likely contains fucoxanthin and not peridinin.

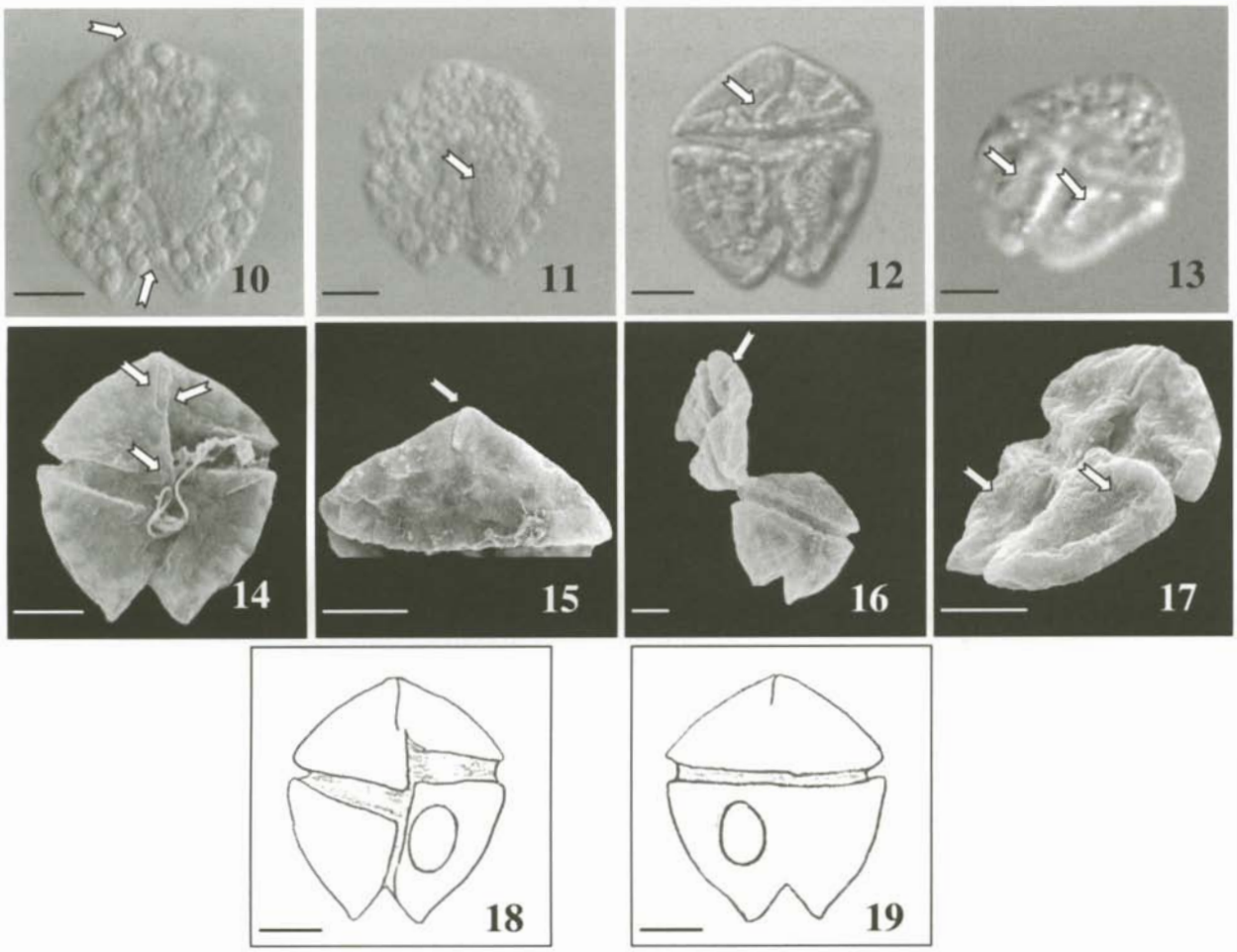
This species has not been associated with any harmful events.

Pairwise distance values obtained from comparison of partial LSU rDNA sequences confirm the status of *K. bicuneiformis* as a new species, with values falling within the range calculated for species of the same genus but not within the range calculated for isolates of the same species (Table 4).

DISCUSSION

The genus *Gymnodinium sensu lato* has recently been divided into four genera (Daugbjerg *et al.* 2000). One of these, *Karenia,* is characterized as having a straight apical groove and fucoxanthin as its major carotenoid. It is readily distinguished from the genus *Karlodinium* by the absence of a ventral pore. *Karenia cristata* and *K. bicuneiformis* are placed within the genus *Karenia* because both species contain a straight apical groove, lack a ventral pore and contain fucoxanthin as the major carotenoid, rather than the more characteristic peridinin of most dinoflagellate species, e.g. *Akashiwo sanguinea* (Hirasaka) Gert Hansen & Moestrup (Daugbjerg *et al.* 2000).

Karenia cristata differs from other *Karenia* species (Table 3) by having an asymmetric hypocone with a larger and more



Figs 10–19. *Karenia bicuneiformis* sp. nov. Scale bar = 10 μm. LM (Figs 10–13); formalin-preserved cells in SEM (Figs 14–17); line drawings (Figs 18, 19).

Fig. 10. Typical cell shape (formalin-preserved) in ventral view showing the w-shaped hypocone (bottom arrow) and the apical groove (top arrow).

Fig. 11. Ventral view of the cell (formalin-preserved), showing the oval shaped nucleus (arrow) on the left-hand side of the cell.

Fig. 12. Ventral view of a live cell, showing the variably shaped chloroplasts (arrow).

Fig. 13. Dorsal view of a live cell, showing the indentations in the right and left lobes of the hypocone (arrows).

Fig. 14. Typical cell shape in ventral view, showing straight apical groove (top arrow), sulcal extension (middle arrow) and the cingulum displacement (bottom arrow).

Fig. 15. Apical groove on the dorsal side of the cell.

Fig. 16. Dorsal view of one cell and the anterior view of the ventral side of another showing clearly the degree of dorso-ventral flattening.

Fig. 17. Ventral view of the indentations in the left and right lobes of hypocone (arrows).

Figs 18, 19. Line drawing of the ventral and dorsal surfaces, respectively.

rounded right lobe and a straight apical groove that is elevated to form an apical crest. These two features are considered the diagnostic features for *K. cristata.* The apical crest present in *K. cristata* is, however, not similar to the much more pronounced carina found in *K. brevis.* The nucleus does not share the dual position and shape of the nucleus of *K. brevisulcata* (Chang 1999) but is large, round to oval and situated in the centre of the cell, with the bulk of the nucleus in the hypocone. Although *K. cristata* is of a similar size to other *Karenia* species, it does not share the circular cell shape of *K. brevisulcata* (Chang 1999) and *K. digitata* (Yang *et al.* 2000). *Karenia cristata* has an apical groove that is long on the ventral side and short on the dorsal side, similar to *K. mikimotoi* (Takayama & Adachi 1984), *K. brevis* (Taylor *et al.* 1995) and *K. digitata* (Yang *et al.* 2000). In this respect it differs from *K. brevisulcata,* which has an apical groove that is short on both ventral and dorsal sides (Chang 1999), and from *K. longicanalis,* which has an apical groove that is long on both the ventral and dorsal sides (Yang *et al.* 2001). The chloroplast shape in *K. cristata* is variable, as it is in *K. mikimotoi* and *K. digitata.* Water discolouration during a bloom of *K. cristata* has been reported as olive-green (Pitcher & Matthews 1996), whereas a coffee-like colour has been reported for *K. mikimotoi* and a yellow-brown colour for *K. brevisulcata* (Chang 1999). As in all *Karenia* species, except *K. longicanalis* (Yang *et al.* 2001), *K. cristata* has a sulcal extension. It shares the same pigments as *K. mikimotoi* (Hansen *et al.* 2000) and *K. brevis* (Millie *et al.* 1995), namely chlorophyll c_2 and c_3, 19′-

butanoyloxy fucoxanthin, fucoxanthin, 19′-hexanoyloxy fucoxanthin, diadinoxanthin, gyroxanthin-diester, chlorophyll *a* and β-carotene, and it also produces an aerosol toxin with harmful properties similar to that of *K. brevisulcata* (Chang 1999) and *K. brevis* (Hemmert 1975; Steidinger & Baden 1984).

New Zealand has the same species, but they have not yet been formally identified. The New Zealand equivalent of *K. cristata* has been referred to as *Gymnodinium* sp. or *Gymnodinium* sp. nov. (cf. *breve*) (Chang 1995; Chang *et al.* 1995), and the equivalent of *K. bicuneiformis* as *Gymnodinium* sp. (Haywood *et al.* 1996). The equivalent of *K. cristata* is similar to *K. cristata* in that it has a crest, a centrally situated nucleus and almost equal cell width and length, and it is dorso-ventrally flattened (< 10 μm deep) (Mackenzie *et al.* 1995). Pairwise distance values obtained from comparison of partial LSU rDNA sequences provide additional evidence for describing *K. cristata* as a new *Karenia* species.

The overall cell size and shape of *K. bicuneiformis* are notably different from those of other *Karenia* species (Table 3). The large, angular and significantly dorso-ventrally flattened cell with a conical epicone and w-shaped hypocone is considered a diagnostic feature. As in *K. brevis* (Takayama 1990) and *K. mikimotoi* (Takayama & Adachi 1984), the nucleus of *K. bicuneiformis* is restricted to the left side of the cell. It is, however, distinguished from the nucleus of *K. mikimotoi* in that it is not reniform and does not extend across the epi- and hypocones (Takayama & Adachi 1984), and it is not round as it is in *K. brevis* (Takayama 1990). The apical groove of *K. bicuneiformis* is long on the ventral side and short on the dorsal side, whereas that of *K. brevisulcata* is short on both the ventral and dorsal sides (Chang 1999). It is also distinguished from *K. longicanalis,* which has an apical groove that is long on both the ventral and dorsal sides (Yang *et al.* 2001). *Karenia bicuneiformis* does not contain an apical crest like *K. cristata* (Chang 1999), nor a very pronounced carina like *K. brevis* (Takayama & Adachi 1984). It has a sulcal extension similar to most *Karenia* species, except *K. longicanalis* (Yang *et al.* 2001). Its chloroplasts are variable in shape – similar to those of *K. cristata, K. mikimotoi* (Takayama & Adachi 1984) and *K. digitata* (Yang *et al.* 2000). Unlike *K. brevisulcata, K. mikimotoi* and *K. brevis,* no toxic effects have been reported for this species.

The *Karenia* sp. present in New Zealand waters, which was referred to as *Gymnodinium* sp., is similar to *K. bicuneiformis* in that it is very large, has a w-shaped hypocone, a nucleus that is situated in the left of the hypocone and is dorso-ventrally flattened by about 10 μm (Haywood *et al.* 1996). Pairwise distance values obtained from comparison of partial LSU rDNA sequences provide additional evidence for describing *K. bicuneiformis* as a new *Karenia* species.

The phylogenetic relationships and character evolution of these two new *Karenia* species and other gymnodinioid species (from the South African coast) from different genera were compared to related species occurring elsewhere in the world and will be published separately.

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