

Is *Karenia* a synonym of *Asterodinium-Brachidinium* (Gymnodiniales, Dinophyceae)?

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From material collected in open waters of the NW and Equatorial Pacific Ocean the detailed morphology of brachidiniaceans based on two specimens of *Asterodinium gracile* is reported for the first time. SEM observations showed that the straight apical groove, the morphological characters and orientation of the cell body were similar to those described for species of *Karenia*. *Brachidinium* and *Asterodinium* showed high morphological variability in the length of the extensions and intermediate specimens with *Karenia*. *Karenia*-like cells that strongly resemble *Brachidinium* and *Asterodinium* but lacking the extensions co-occurred with the typical specimens. The life cycle and morphology of *Karenia papilionacea* should be investigated under natural conditions because of the strong similarity with the brachidiniaceans.

Key words: Phytoplankton, *Asterodinium*, *Brachidinium*, *Brachydidinium*, *Gymnodinium*, *Karenia*, Dinophyta, apical groove, SEM, Pacific Ocean.

Introduction

Fixatives, such as formaline or Lugol, do not sufficiently preserve unarmoured dinoflagellates to allow species identification. Body shape and morphology often change during the process of fixation so that even differentiating between the genera *Gymnodinium* Stein and *Gyrodinium* Kofoid *et* Swezy is difficult (ELBRÄCHTER 1979). Most of the original descriptions, often based on fixed specimens, were insufficiently detailed and the morphological characters for the differentiation of the species were sparse. TAKAYAMA (1985) developed a scanning electron microscopy (SEM) technique that allowed the observation of the apical grooves (acrobases) in some gymnodinioid cells. A revision by DAUGBJERG *et al.* (2000) combined large subunit (LSU) rDNA sequences, ultrastructure and chloroplast

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pigment composition, which allowed for the division of the large heterogeneous genus *Gymnodinium* into four genera: *Gymnodinium* sensu stricto, with a horseshoe-shaped apical groove and peridinin as the main carotenoid; *Akashiwo* G. Hansen *et* Moestrup, with a clockwise spiral apical groove and peridinin; *Karenia* G. Hansen *et* Moestrup, with a straight apical groove and fucoxanthin; and *Karlodinium* J. Larsen, with a short straight apical groove, a ventral pore, and fucoxanthin. Later the genus *Takayama* de Salas, Bolch, Botes *et* Hallegraef, with sigmoid apical grooves, was also erected (DE SALAS *et al.* 2003). Since then, the description of new species of the genus *Karenia* has proliferated (e.g., BOTES *et al.* 2003; HAYWOOD *et al.* 2004). Unialgal cultures have been established by isolation of vegetative cells from coastal waters and maintained under laboratory conditions. The abundant material available allows detailed studies on the ultrastructure, pigment and toxin composition and molecular phylogeny. Little is known on the morphology and the life cycle of these species under natural conditions.

There are few records of gymnodinioid cells identified at the species level in open waters. Morphological characters such as the occurrence of body extensions facilitate identification under routine analysis in taxa such as *Gyrodinium falcatum* Kofoid *et* Swezy (= *Gymnodinium fusus* Schütt pro parte). Observations of live specimens of *G. falcatum* from natural samples (ELBRÄCHTER 1979; YOSHIMATSU 1990) or temporal cultures (KONOVALOVA 2003) showed high morphological variability and fast changes of cell shape and length of extensions. Stages of the life cycle of *G. falcatum* were described as separate species, such as *Pseliodinium vaubanii* Sournia (KONOVALOVA 2003).

The occurrence of extensions is the main character of the unarmoured genera *Brachidinium* F.J.R Taylor and *Asterodinium* Sournia, which species were described from single or a few preserved specimens. The only known morphological characters of the brachidiniaceans were the pronounced compressed cell body, yellow-green chloroplasts and the prominent nucleus. Despite the lack of morphological data, the order Brachidiniales A.R. Loeblich III *ex* Sournia was established exclusively for these genera. No photomicrographs of *Asterodinium* were available in the literature. From Mediterranean, Atlantic and Pacific waters, GÓMEZ (2003) reported a high variability in the length of the extensions among the specimens of *Asterodinium*. Within this context, GÓMEZ (2003, p. 339) concluded: »Further research should address the *Asterodinium gracile* complex; does it constitute one species with high morphological variability, depending on environmental conditions, or are different species involved?«

Brachidinium is more commonly recorded than *Asterodinium* and one section plot of its distribution is even available (MARGALEF 1975). *Brachidinium* also showed a high variability in the length and relative position of the extensions, being often reported as *Brachidinium* sp. because the specimens cannot be ascribed to any known species. No detailed study on the morphological characters of *Brachidinium* was available. From Lugol-fixed specimens and one live specimen, GÓMEZ *et al.* (2005) reported details of the morphology of *Brachidinium*. SOURNIA described 4 of the 5 species of brachidiniaceans. From the same samples of his records, SOURNIA illustrated gymnodinioid cells that he considered close to *Brachidinium* (SOURNIA 1972, p. 157). Later SOURNIA (1986) hypothesized that the Brachidiniales constitute a part of the life cycle of more common dinoflagellates.

Despite the scarcity of specimens of brachidiniaceans available and the delicacy of them, by using the TAKAYAMA'S method, we have successfully obtained the first SEM pic-

tures of a member of the order Brachidiniales based on two specimens of *Asterodinium gracile* Sournia. The morphological characters of *Asterodinium* were similar to those of some species of the genus *Karenia*. Gymnodinoid cells which appearance strongly resembled specimens of *Brachidinium* and *Asterodinium* but lacking the extensions co-occurred with the typical *Brachidinium* and *Asterodinium*. The life cycle and the morphology of some species of *Karenia* should be investigated under natural conditions because they may correspond to forms of *Brachidinium-Asterodinium*.

Material and methods

Sample collection and light microscopical observations were as in GÓMEZ et al. (2004, 2005). For SEM, specimens were isolated with a capillary from sedimentation chambers and adhered to poly-L-lysine-coated cover-slip. Fixed cells attached to the cover-slip were rinsed twice in distilled water for 5 min each. Cells were then dehydrated through an ethanol series, transferred into isoamyl acetate (TAKAYAMA 1998), dried in a critical point drier (HCP-2, Hitachi, Japan), and coated with Au-Pd. Observations were made using SEM (S-430 and S-800, Hitachi, Japan).

Results and discussion

Asterodinium

The new records of *Asterodinium* from the western Pacific Ocean (Tab. 1) showed a high variability in the length of the extensions as previously reported by GÓMEZ (2003). Observations with Nomarski differential interference contrast (DIC) optics showed the transverse flagellum; however the insertion point was not clearly revealed (Figs. 1, 2). With SEM, the insertion point was observed in two specimens of *Asterodinium gracile* (Figs. 8–15). The ventral view corresponded to the nucleus in the left hyposome. The me-

Tab. 1. Records of *Asterodinium* from the Pacific Ocean. Date, depth (m), geographic coordinates (latitude, longitude), and dimensions: width at the level of the cingulum (μm); total length (μm) of each record. Records from the vicinity of the Kuroshio Current can be found in GÓMEZ (2003). *Karenia*-like cells are omitted.

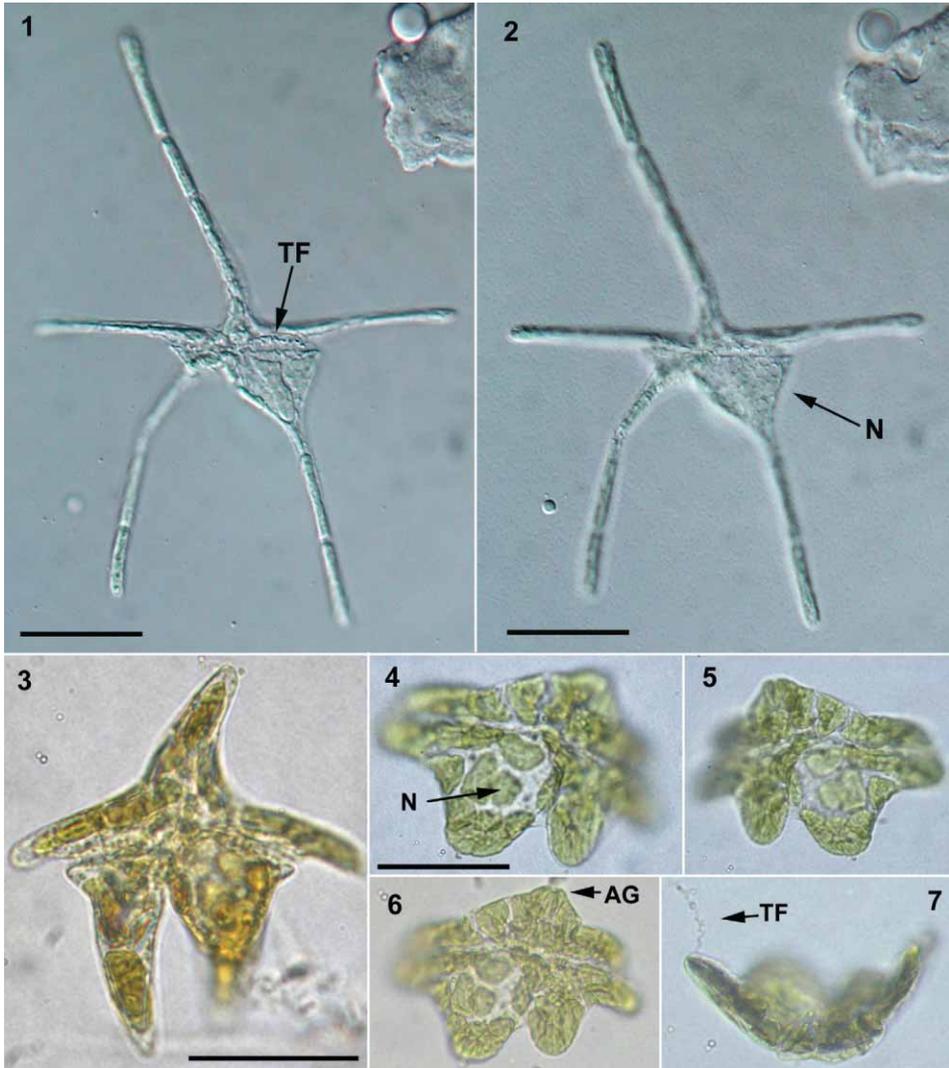
Taxon	Date	Depth	Latitude	Longitude	Wide	Length
<i>Asterodinium gracile</i>	16 Nov 2002	50	5°11'N	124°05'E	25	65
<i>Asterodinium gracile</i> (Figs 8–15)	16 Nov 2002	50	5°11'N	124°05'E	23	55
<i>Asterodinium gracile</i> (Fig. 3)	17 Nov 2002	30	5°N	121°E	28	55
<i>Asterodinium gracile</i>	17 Nov 2002	75	5°N	121°E	23	60
<i>Asterodinium gracile</i>	19 Nov 2002	75	7°25'N	121°12'E	20	100
<i>Asterodinium gracile</i>	19 Nov 2002	50	7°25'N	121°12'E	24	105
<i>Asterodinium gracile</i>	03 Dec 2002	30	8°50'N	121°48'E	20	–
<i>Asterodinium gracile</i>	18 Jan 2003	120	0°	170°E	21	110
<i>Asterodinium gracile</i>	17 Jan 2003	100	0°	165°E	22	60
<i>Asterodinium</i> sp. (Figs 4–7)	23 Jan 2003	120	0°	170°W	32	–

dian cingulum was descending and displaced by the cingulum width (Figs. 10, 14). The longitudinal flagellum was not observed with SEM, having probably been lost during sample preparation. The cavity that appeared below the terminal part of the transverse flagellum might correspond to the place of the insertion of the longitudinal flagellum (Figs. 11, 15). A vertically oriented cingular ridge runs between the two points of flagellar insertion (Fig. 15). The ventral ridges are present in species of *Karenia* and have an intercingular tubular structure that traverses the proximal and distal ends of the cingulum (HAYWOOD et al. 2004). The sulcal intrusion on the epitheca of *A. gracile* is open and extends to left of the apex (Fig. 14). A straight groove with rolled margins was visible in the central apical extension of both specimens (Figs. 9, 13). The sulcus was not well defined under SEM (Figs. 11, 15). These morphological characters were similar to those described for *Karenia papilionacea* Haywood et Steidinger or *K. bicuneiformis* Botes, Sym et Pitcher (= *K. bidigitata* Haywood et Steidinger) (BOTES et al. 2003; HAYWOOD et al. 2004). In *Asterodinium gracile* the carina or apical protrusion is extremely elongated and is named the central apical extension (Fig. 23). With DIC and SEM, trichocysts were observed in *Asterodinium gracile*, sometimes forming clusters. SEM observations showed that the basal part of the trichocysts was thicker than the terminal hair (Fig. 13). The species *Asterodinium spinosum* Sournia was described from a single fixed specimen based on the occurrence of two small spines in the central apical extension (SOURNIA 1972). However, the present study reveals that these »spines« may be the thick basal part of the fragile trichocysts. *Asterodinium spinosum* should not be considered a species separate from the type species.

One of the specimens of *Asterodinium* showed short extensions, being an intermediate between the typical *Asterodinium* and the *Asterodinium* that lacks any extensions (Fig. 3). Strongly dorso-ventrally compressed gymnodinoid cells with short lobulate extensions were observed showing the distinctive chloroplasts, the straight apical groove and the prominent nucleus of *Asterodinium* (Figs. 4–7).

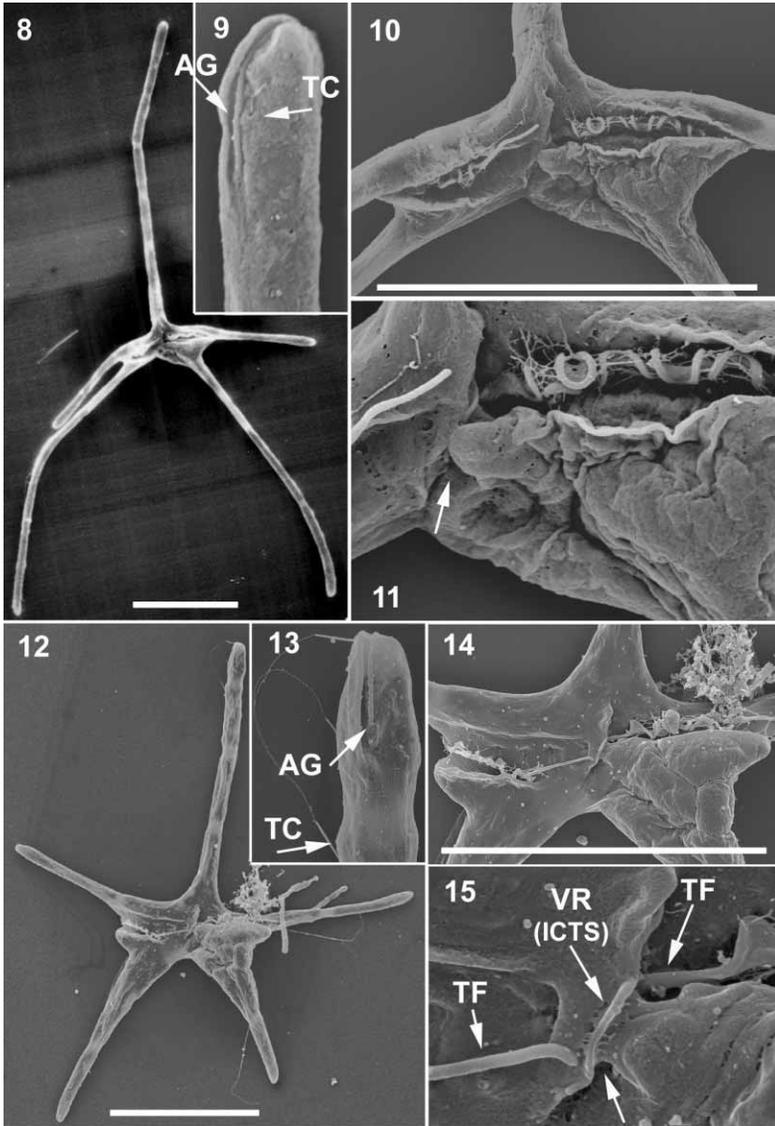
Brachidinium

Light microscopy observations of Lugol-fixed specimens and one live specimen of *Brachidinium capitatum* showed morphological characters such as a short straight apical groove (Fig. 20). The orientation and morphology of the cell body were similar to those in *Asterodinium* (Figs. 16, 18, 20), although the details of the intercingular region were not clearly visible. Several of the specimens of *B. capitatum* showed a darker area in the right hyposome (Figs. 16, 18, 26). This region appeared with a variable size and colour, often similar to the brown colour of the nucleus of the Lugol-fixed specimens. After DAPI-staining this region fluoresced when excited with UV light but less bright than the dinokaryon. GÓMEZ et al. (2005) interpreted this darker area as a secondary nucleus in *B. capitatum* as occurred in some freshwater dinoflagellates. However this region varied in size, shape and colour among the Lugol-fixed specimens (Figs. 16, 18) more than would be expected for a secondary nucleus. The occurrence of an accumulation body in the right hyposome of *B. capitatum* appeared a more probable explanation. The role of the accumulation bodies in dinoflagellates is poorly understood. It is believed to be in an endocytic pathway functioning as a lysosome (ZHOU and FRITZ 1994). Among the species of *Karenia*, *K. papilionacea* was also characterised by an accumulation body that was visible in the right hyposome in 10% of cells under culture (HAYWOOD et al. 2004).



Figs. 1–7. DIC photomicrographs of the ventral view of *Asterodinium gracile* in different focuses (30°N; 138°E, 80 m depth). See the nucleus in the left hyposome and the transverse flagellum. Figs. 3–7. LM micrographs of *Asterodinium*. Fig. 3. Specimen of *Asterodinium* in ventral view with short extensions from the Celebes Sea. Figs. 4–7. Specimen of *Asterodinium* with lobulate extensions from the Central Equatorial Pacific. Figs. 4, 6. Dorsal views. Figs. 5, 7. Ventral and lateral views respectively. The arrows point to the nucleus, transverse flagellum and the apical groove (acrobase). AG=apical groove; TF= transverse flagellum; N=nucleus. Scale bars = 20 μ m.

Large extensions are one of the main characteristics of the Brachidinales. Specimens lacking the extensions are hard to assign to *Brachidinium* or *Asterodinium* and are consequently pooled as unidentified gymnodinioid cells under routine microscopical analysis of fixed phytoplankton. In the present study gymnodinioid cells that strongly resembled



Figs. 8–15. SEM pictures of two specimens of *Asterodinium gracile* in the ventral view. Figs 8–11. Specimen with long extensions collected from the Kuroshio Current region (33° 30'N; 138°E, 100 m depth). Fig. 9. Detail of the central apical extension. See the straight apical groove or acrobase and the trichocyst pores. Figs. 10–11. Detail of the central body and the location of the insertion of the transverse flagellum. Fig. 11. The arrow points to a cavity where the longitudinal flagellum might arise. Figs. 12–15. Specimen with shorter extensions collected from the Celebes Sea (5°11'N; 124°05'E, 50 m depth). Fig. 13. Detail of the central apical extension. See the apical groove and trichocysts. Figs. 14–15. Detail of the central body. Fig. 15. A ventral ridge runs between the two points of flagellar insertion. The arrow below the ventral ridge points to a cavity where the longitudinal flagellum might arise. AG=Apical groove; TF=transverse flagellum; TC=trichocyst; VR=ventral ridge; ICTS=Intercingular tubular structure. Scale bars = 20 μ m.

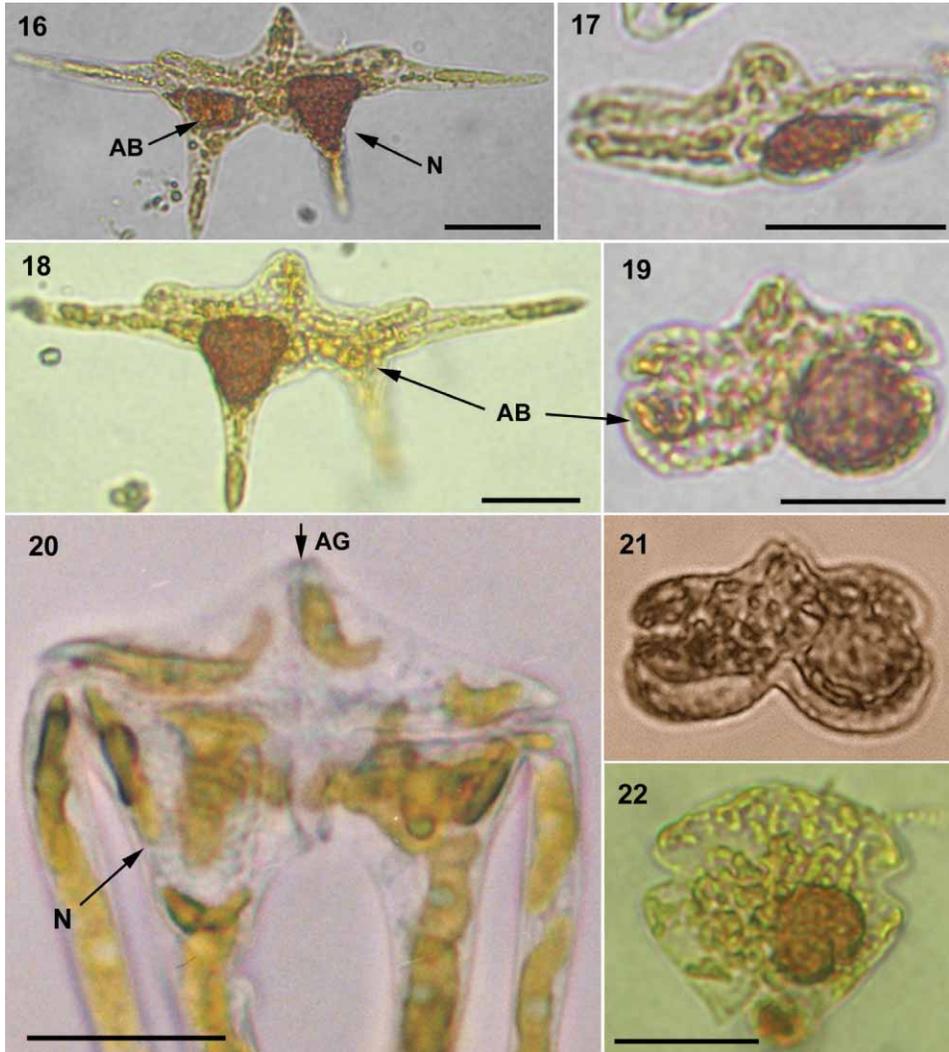
B. capitatum cells lacking the extensions co-occurred with the typical *B. capitatum* (Figs. 17, 19, 26). Several of the typical *B. capitatum* showed accumulation body of variable size and colour in the right hyposome (Figs. 16, 18), which was also visible in the specimens lacking the extensions (Fig. 19). These gymnodinioid cells were also reported in previous studies including records of *B. capitatum*. In the SW Indian Ocean, SOURNIA (1972) described two new species of *Brachidinium* and also found the type species with variable morphology. From the same samples, SOURNIA illustrated gymnodinioid cells that he considered close to *Brachidinium* (Fig. 25). Observations of live specimens of *Brachidinium* (LÉGER 1971; GÓMEZ et al. 2005) have even showed the extensions to be moveable. In cultures, the carina of *Karenia papilionacea* contracts forward when the cells are stressed (HAYWOOD et al. 2004, p. 170). SOURNIA (1972, p. 157) also illustrated the gymnodinioid cell folded with the carina contracted forward (Fig. 25). *Brachidinium capitatum* (Fig. 26) and *K. papilionacea* co-occur in the coastal waters of the south of Japan (misidentified as *Karenia brevis* (Davis) G. Hansen et Moestrup) and in other warm to temperate waters (IZUKA 1976; HAYWOOD et al. 2004). From the eastern Mediterranean Sea, ABBOUD-ABI SAAB (1989) found *B. capitatum* and also illustrated several unidentified gymnodinioid cells with similar morphology to the *Karenia*-like cells reported in SOURNIA (1972) and in the present study (Figs 17, 19). *Karenia papilionacea* and *B. capitatum* are both cosmopolitan taxa that appear in surface waters in low abundance (MARGALEF 1975; HAYWOOD et al. 2004).

In the table 2 the morphological and ecological characters of *B. capitatum* and *K. papilionacea* have been compared. The distinctive morphological characters of *K. papilionacea* coincided with *B. capitatum* (Tab. 2, Figs 16–20). The *Karenia*-like cells that co-occurred with *B. capitatum* showed morphology similar to that of the Lugol-fixed cells of the culture of *K. papilionacea* at the Cawthron Institute, New Zealand (Fig. 21).

According to HAYWOOD et al. (2004), *Karenia bicuneiformis* (= *K. bidigitata*) often co-occurs with *K. papilionacea*. In the present study several specimens were tentatively identified as *K. bicuneiformis*, also co-occurring with *B. capitatum* and the *Karenia*-like cells (Fig. 22). *Karenia papilionacea* and *K. bicuneiformis* are very close from morphological and phylogenetical points of view (HAYWOOD et al. 2004).

No studies are available on the projection of the cell body extensions in unarmoured dinoflagellates. ZIRBEL et al. (2000) reported that the length of the extensions of the armoured dinoflagellate *Ceratocorys horrida* Stein varied as an adaptive strategy according to water motion. Specimens lacking the extensions appeared as early as 1 h after an increase in turbulence. Cells with long extensions were transforming into cells with short or no extensions. This phenomenon was reversible and the extensions reappeared after a reduction in turbulence (ZIRBEL et al. 2000).

The expansion of the cell body with the projection of extensions is expected to be easier for unarmoured dinoflagellates. Beyond the high shape plasticity with fast contractions of the carina of *K. papilionacea*, a high variability in cell size in cultures is also observable. HAYWOOD et al. (2004, p. 175) reported, »Cellular size measurements are given as ranges, reflecting small to large cells present in cultures that were not separated into size classes because of the intergradations between sizes and because the significance of the size classes cannot be addressed until the life cycle of these species is known«.

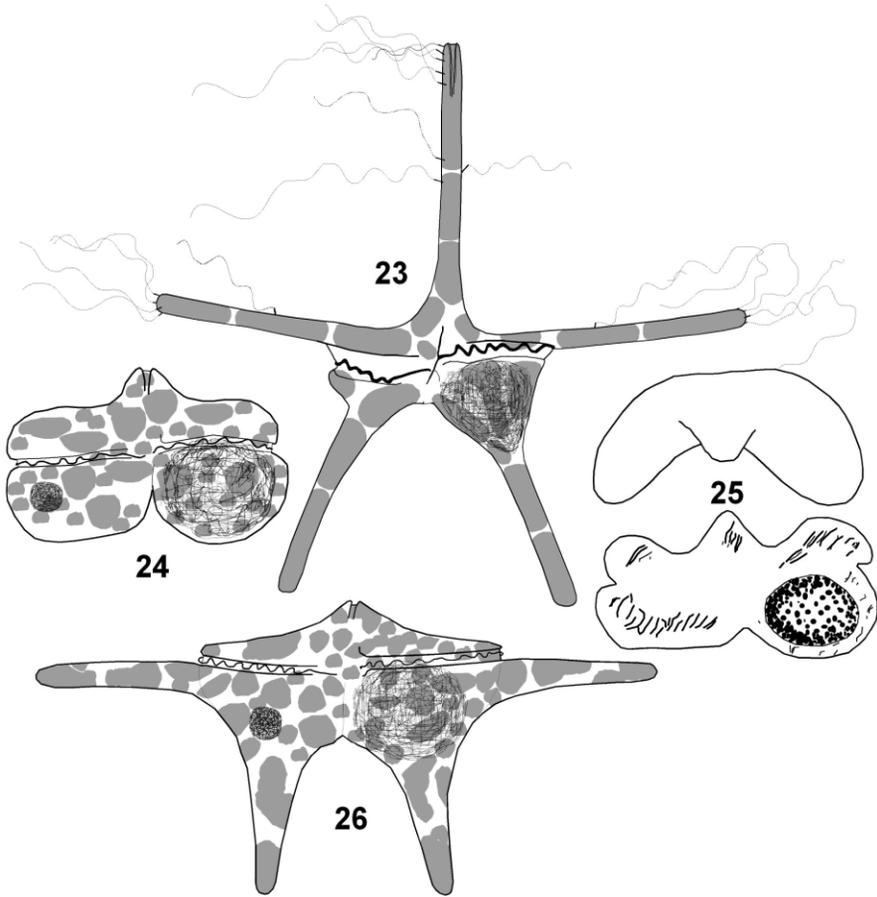


Figs. 16–22. LM micrographs of *Brachidinium capitatum* with and lacking the extensions, *Karenia papilionacea* and *K. cf. bicuneiformis* (= *K. bidigitata*). All Lugol-fixed specimens except Fig. 20. Fig. 16. Ventral view of a specimen of *Brachidinium* with a prominent accumulation body from the Philippine Sea (32°N; 138°E, 30 m depth). Fig. 17. *Karenia*-like cell that strongly resembles *Brachidinium* but lacking the extensions (32°N; 138°E, 80 m depth). Fig. 18. Dorsal view of a specimen of *Brachidinium* with a less marked accumulation body (0°; 160°E, 0 m depth). Fig. 19. *Karenia*-like cell from the same sample as figure 16 with an accumulation body (32°N; 138°E, 30 m depth). Fig. 20. Back focus of the cell body of a live specimen of *B. capitatum* in the dorsal view (see GÓMEZ et al. 2005). Fig. 21. Lugol-fixed specimen of *K. papilionacea* from the culture of the Cawthron Institute, New Zealand. Fig. 22. Tentatively *Karenia bicuneiformis* (= *K. bidigitata*) (0°; 175°E, 15 m depth). AB=accumulation body; AG=Apical groove; N=nucleus. Scale bars = 20 μ m.

Tab. 2. Comparative morphological and ecological characteristics of *Brachidinium capitatum* (Lugol-fixed specimens from open waters and one live specimen from the coastal waters of Japan). Data from *Karenia papilionacea* (from cultures) based on HAYWOOD et al. (2004).

Character	<i>Brachidinium capitatum</i>	<i>Karenia papilionacea</i>
Apical groove	Straight, very short and bisects the carina	Straight, very short and bisects the carina
Carina (apical protrusion)	Pointed	Pointed
Dorsoventral compression	Moderate	Moderate
Nucleus shape	Spherical to slightly oval	Spherical to slightly oval
Nucleus location	Left hyposome	Left hyposome
Hyposome shape	Bilobed, centrally excavated	Bilobed, centrally excavated
Hyposome excavation	Pronounced	Pronounced
Cell width at the cingulum level	25–55 μm	18–48 μm
Cingulum	Median, descending and displaced by the cingulum width	Median, descending and displaced by the cingulum width
Sulcal intrusion on the epitheca	No visible from Lugol-fixed specimens	Open sulcus extends to left of apex
Chloroplast number	Many	Typically 2–20 chloroplasts per cell
Chloroplast shape	Round to reniform in cell body, elongate plastids in the extensions	Round to reniform
Chloroplast colour	Yellow-green	Yellow-green
Fucoxanthin	No tested	yes
Accumulation body occurrence	~25% of the specimens in nature	10% of the specimens in culture
Accumulation body number	1	1
Accumulation body location	Right hyposome	Right hyposome
Accumulation body shape	Spherical to oval	Spherical to oval
Accumulation body size	Variable	Variable
Cell movements	Carina and extensions change of shape and length	Carina contracts forward to overlap the epitheca
Geographical distribution	Cosmopolitan (temperate to warm)	Cosmopolitan (temperate to warm)
Ecological distribution	Surface, coastal to open waters (MARGALEF 1975)	Surface, coastal to open waters
Abundance in natural waters	Low (MARGALEF 1975)	Low (<1000 cells L^{-1})
Period of max. abundance	Summer	Summer
Life cycle	Unknown	Unknown

The life cycle and morphology of species such as *K. papilionacea* should be investigated under natural conditions because they may correspond to a life stage of brachidiniaceans. There are no reasons to retain the order Brachidinales because the morphological characters of *Brachidinium-Asterodinium* do not differ from those in *Karenia*.



Figs. 23–26. Line drawings of *Asterodinium*, *Brachidinium* and the related *Karenia* cell. Fig. 23. Typical *Asterodinium gracile*. Fig. 24. *Karenia*-like cell related to the brachidiniaceans. Fig. 25. Two views of the *Karenia*-like cell related to *Brachidinium* according to SOURNIA (1972, p. 157). Fig. 26. Typical *Brachidinium capitatum*.

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