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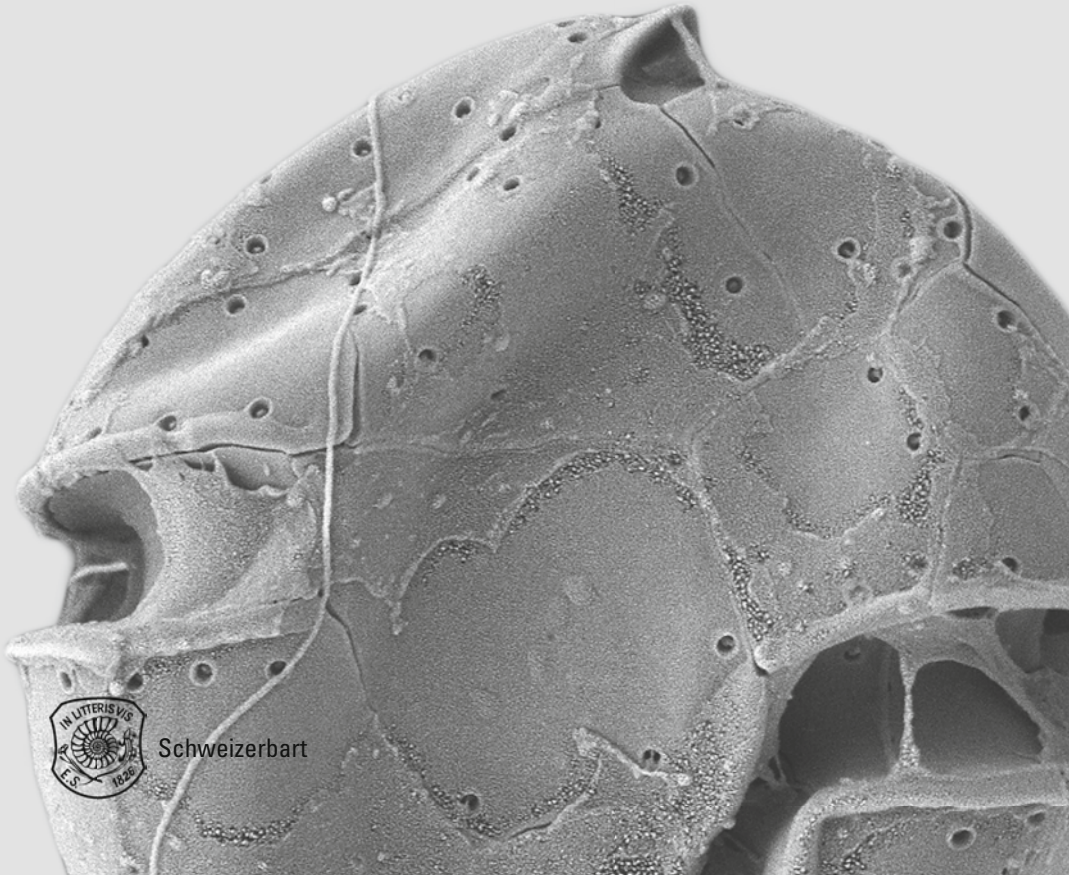
Mona Hoppenrath, Shauna A. Murray, Nicolas Chomérat, Takeo Horiguchi

Marine benthic dinoflagellates

– unveiling their worldwide biodiversity



Schweizerbart

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Impressum

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Volume 54: Marine benthic dinoflagellates – unveiling their worldwide biodiversity
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Front cover: *Herdmania*, the taxon was named to honor E.C. Herdman, who did the pioneering studies about marine sand-dwelling dinoflagellates (1921–1924).

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Kommetjie, Cape Town, South Africa. Diverse habitats at one site: sandy beach, rocky shore containing tide pools, and floating macroalgae.

Greetings

At present fewer than two million species are known to inhabit the biosphere, but experts estimate that between 5 to 50 times as many species are actually living on our planet. The relatively unexplored deep sea is fascinating for the public by its unknown biodiversity. But there is no need to search those far reaches to discover new species, they can be found "right in front of the door". To understand marine habitats, a lot of effort has been put into phytoplankton inventories worldwide. In particular, Harmful Algal Blooms (HABs) caused by diverse dinoflagellate taxa, are a major, socially and economically relevant field of research. In recent years the importance of benthic HABs is increasingly recognized because of the impact of ciguatera, which is the most important food borne disease of non-bacterial origin in the world and is caused by benthic dinoflagellate species. Benthic dinoflagellates are understudied, and the known species diversity has nearly doubled in the past 15 years, with new taxa discovered every year – including new genera. This book is the first comprehensive summary of their worldwide biodiversity and biogeography, covering a total of 189 species in 45 genera. With its excellent illustrations it will certainly help to identify and monitor these species and to assess potential risks of HABs causes by some of them. Hopefully, this book will also broaden the awareness of these fascinating, tiny, single-celled marine organisms and motivate students to study them.

The authors, who are among the very few expert taxonomists for these dinoflagellates (responsible for over a third of the taxon descriptions), illustrate through their long-term research that systematics and compiling inventories of life is a demanding and complex science requiring many years of experience and patience as well as advanced laboratory techniques.

My congratulations go to the four authors of this timely and important monograph, which certainly will serve as a standard work for many years to come. Senckenberg is proud to have supported this great project.

Volker Mosbrugger
Senckenberg Gesellschaft für Naturforschung

Foreword

It is a pleasure to introduce *Marine benthic dinoflagellates – unveiling their worldwide biodiversity*. The complicated taxonomy of benthic dinoflagellates is summarized using the most recent information from combinations of detailed microscopic observations, genetic approaches and careful, patient field studies. This work provides new and useful clues on the biogeography, systematics and ecology of this group, including some of the organisms causing harmful outbreaks, and concurrently highlights the unresolved difficulties and challenges for the thorough comprehension of the benthic dinoflagellates. This effort benefitted not only from recent technological advances, but especially, from the youth and diversity (from Germany, France, Australia and Japan) of the co-authors. The expertise of these young, motivated researchers holds much promise for the future of dinoflagellate taxonomy. At the beginning of the XXIst century, taxonomy is essential not only to establishing the worldwide biodiversity of benthic dinoflagellates, but to identify particular harmful taxa. Indeed, a main aim of this effort is to help monitoring programs prevent and mitigate the consequences of harmful events affecting human and ecosystem health. Finally, the thorough treatment of the benthic dinoflagellates provided in this book constitutes a solid basis for future studies on the structure and dynamics of benthic dinoflagellate communities.

This publication is especially timely because it comes to press in the spring of 2014, ten years after Professor Ramon Margalef passed away. Margalef would be particularly delighted reading it given his special admiration for dinoflagellates, as he clearly expressed in his contribution to the VIIIth Conference on Harmful Algae" held in Vigo on 1997: "*Dinoflagellates are admirable in their organization and behaviour*" (Margalef 1997). This book provides excellent images of this wonder of nature. The high quality and resolution of the microphotographs illustrate what it would be defined in Margalef's terms as "a comprehensive dictionary" of benthic dinoflagel-

lates or using the author's words, "the unveiled worldwide biodiversity", of this group. As was Margalef, we are certain the authors have experienced the pleasure of observing nature and the major gratification will be to communicate the fruits of the long hours of meticulous and inspired work. More importantly, this book will introduce scientists to the beauty, complexity, and importance of dinoflagellates for generations to come.

We congratulate M. Hoppenrath, S.A. Murray, N. Chomérat and T. Horiguchi on their fine publication. It is certain this volume will be a success and we hope that it will not be the last joint effort to bring the heretofore neglected benthic dinoflagellates to the forefront.

Elisa Berdalet, Raphael Kudela, Patricia A. Tester
February 2014

Contents

Acknowledgements

This book was a "project" for Mona Hoppenrath (MH) but all co-authors had to work on it alongside, so that we want to thank our colleagues and students for their patience during busy writing times.

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I. Introduction

The first studies of dinoflagellates inhabiting in sandy sediments were conducted early last century (Kofoid and Swezy 1921, E.C. Herdman 1922, 1924a, b, Balech 1956), however, few studies were conducted in the decades after these. Further investigations started in the 1980s (e.g. Saunders and Dodge 1984, Larsen 1985, Dodge and Lewis 1986, Horiguchi and Pienaar 1988a, Horiguchi 1995, Faust 1995). Faust and Horiguchi had a continuous interest in benthic dinoflagellates, exploring mangrove and coral reef habitats and tide pools (e.g. Faust 1993a, b, 1997, 1999, Horiguchi and Chihara 1983a, 1988, Horiguchi and Pienaar 1994a, Horiguchi et al. 2000, 2011, 2012). Comprehensive studies of sand habitats occurred in the 2000s (Hoppenrath 2000b, Murray 2003, Tamura 2005, Mohammad-Noor et al. 2007b, Al-Yamani and Saburova 2010). These studies showed that a species composition quite distinct from planktonic habitats was present in benthic habitats. Less than 10% of the about 2000 described extant dinoflagellate species appear to be benthic (Taylor et al. 2008). They occur in different types of habitats (see chapter II) and appear to be adapted to a benthic life style in their morphology, in their behavior, and some also in their life cycles (see ecology chapter VI).

Some taxa are known to produce toxins impacting humans, particularly those occurring in tropical and subtropical regions (see chapter VII), which has caused an increase in research interest in benthic dinoflagellates. The study of harmful benthic dinoflagellates started in late 1970s with the discovery that a benthic species, later named *Gambierdiscus toxicus*, was thought to be responsible for ciguatera fish poisoning, a type of human poisoning linked to the consumption of certain species of tropical reef fish (Yasumoto et al. 1977). As ciguatera fish poisoning incidences are increasing, and the distribution of toxin producing benthic taxa seems to expand, an understanding of the species diversity and their identification is becoming more and more important. Blooms of harmful benthic dinoflagellates can cause serious human and environmental health problems. Recently the potentially toxic species have been subject of intense research activities (e.g. Litaker et al. 2009, Laza-Martínez et al. 2011; reviews: Parsons et al. 2012, Hoppenrath et al. 2013a).

The lack of comprehensive taxonomic investigations of benthic dinoflagellates complicates progress in our understanding of their biodiversity, biogeography and ecology, and motivated us to compile current information into this book. One hundred and eighty-nine species belonging to 45 genera are described and their known distribution recorded herein. The distribution section for the species lists the references in the following order: Arctic Ocean, North Atlantic (e.g., UK, North Sea, France, Spain, Portugal, east USA, Gulf of Mexico, Caribbean Sea), South Atlantic (e.g., Cape Town, South Africa), Mediterranean Sea,

Arabian/Persian Gulf, Indian Ocean (e.g., Viet Nam, Malaysia, West Australia, South Africa), North Pacific (e.g., Sea of Japan, Korea, Japan, BC Canada, California), South Pacific (e.g., East Australia, New Caledonia, French Polynesia, New Zealand). It is the first comprehensive treatise on the group, and it is our intention that it will facilitate further studies.

The classification of dinoflagellates is currently changing and is far from being settled, with the discovery of new species and genera, and the rearrangements of systematic entities. Many benthic dinoflagellate genera have unusual morphologies and appear to be not closely related to known planktonic taxa, and molecular phylogenetic analyses frequently show low statistical support for any relationship (see chapter IV). They show unique thecal plate arrangements when compared to planktonic species, e.g. *Adenoides, Amphidiniella, Cabra, Planodinium, Rhinodinium, Sabulodinium* (see taxonomy chapter III). Therefore, no higher classification was used in this book and the genera (and species within a genus) are presented in alphabetical order. No keys were provided but information about similar species with which a taxon can be confused is given.

A good introduction to dinoflagellates is the Tree of Life web project page (http://tolweb.org/Dinoflagellates/2445). Summaries of main dinoflagellate characteristics were published in Hoppenrath et al. (2009a, 2013a) and of their diversity in F. J. R. Taylor et al. (2008). The cell orientation is explained in figure 1. For the thecal plate designation, the Kofoid system as modified and described in Fensome et al. (1993) was followed (Fig. 2). Some benthic taxa have thecal tabulations difficult to interpret and sometimes different designations (plate formulae) have been published for one taxon.

As our understanding of the morphological and genetic diversity of dinoflagellates has increased in recent years, some original descriptions of species may no longer be adequate to identify a taxon. Cryptic species diversity has been detected already (Murray et al. 2012), and it is highly likely that further cryptic species will be found. Furthermore, some old taxonomic concepts are no longer valid. For example within the unarmoured (athecate, naked) dinoflagellate genera some genus delimitations are unsatisfactory, and reclassification is still ongoing. For instance, the genera *Amphidinium*, *Gymnodinium*, and *Gyrodinium* were redefined (Daugbjerg et al. 2000, Flø Jørgensen et al. 2004a, Murray et al. 2004). As a consequence of the redefinitions many species can no longer be classified in the genera, need reinvestigation, reclassification or classification within new genera. For practical reasons and not to make them "nameless", the old generic names were used in this book, and the genera were separated into sensu stricto (s.s.) and sensu lato (s.l.) species.

Dinoflagellates are protists that historically have been treated in accordance with the International Code of Botanical Nomenclature (ICBN) and the International Code of Zoological Nomenclature (ICZN) – ambiregnal taxa. It has been agreed on solely applying the botanical code for dinoflagellates in future, and we here follow the latest version of the International Code of Nomenclature (ICN) for algae, fungi, and plants – the Melbourne Code (McNeill et al. 2012). Some species epithets (names) have been corrected, following article 32.2: "Names or epithets published with an improper Latin termination but otherwise in accordance with this *Code* are regarded as validly published; they are to be changed to accord with Art. 16–19, 21, 23, and 24, with-

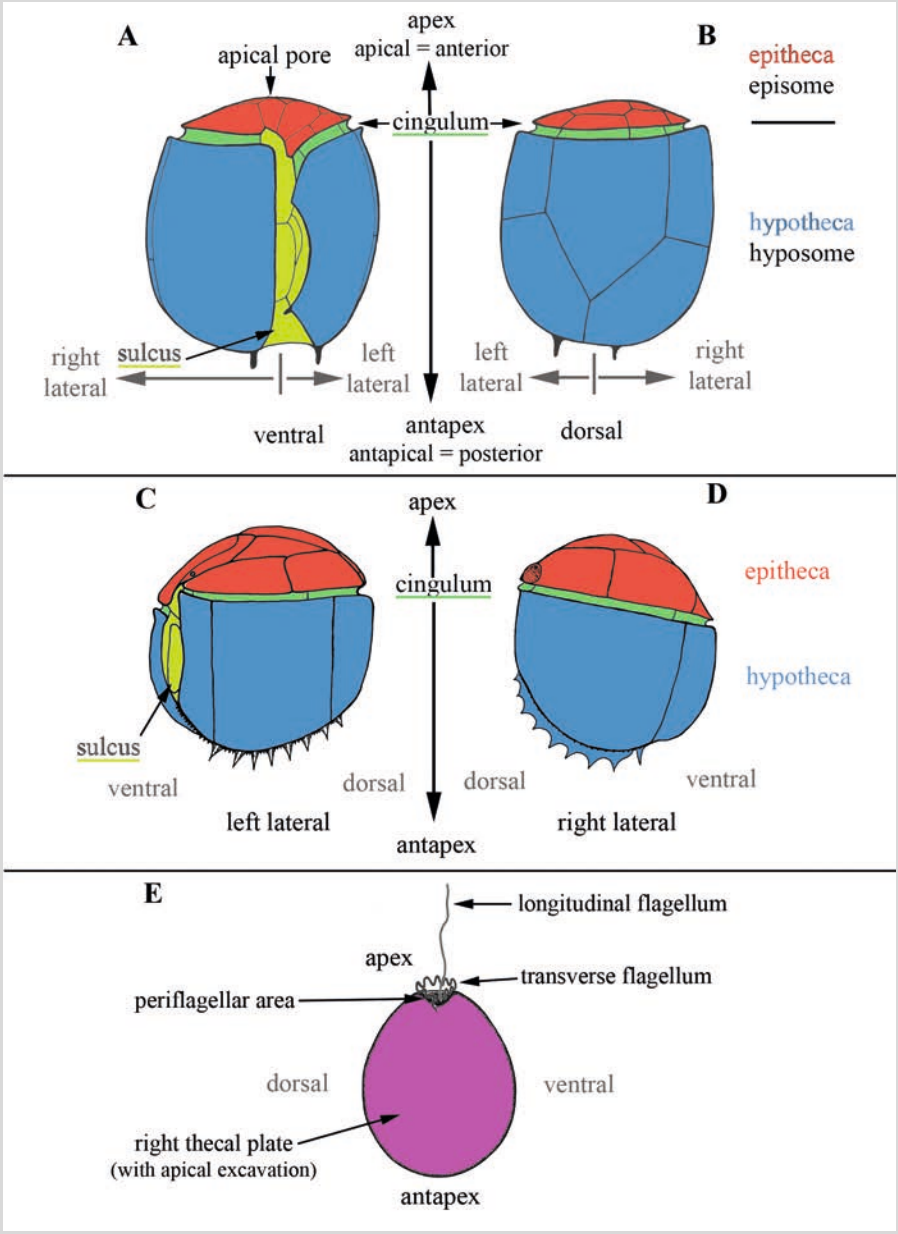


Fig. 1: Cell orientation. A–D: Dinokont cells. A, B: Dorsoventrally flattened cell. C, D: Laterally flattened cell. E: Prorocentroids, desmokont cell.

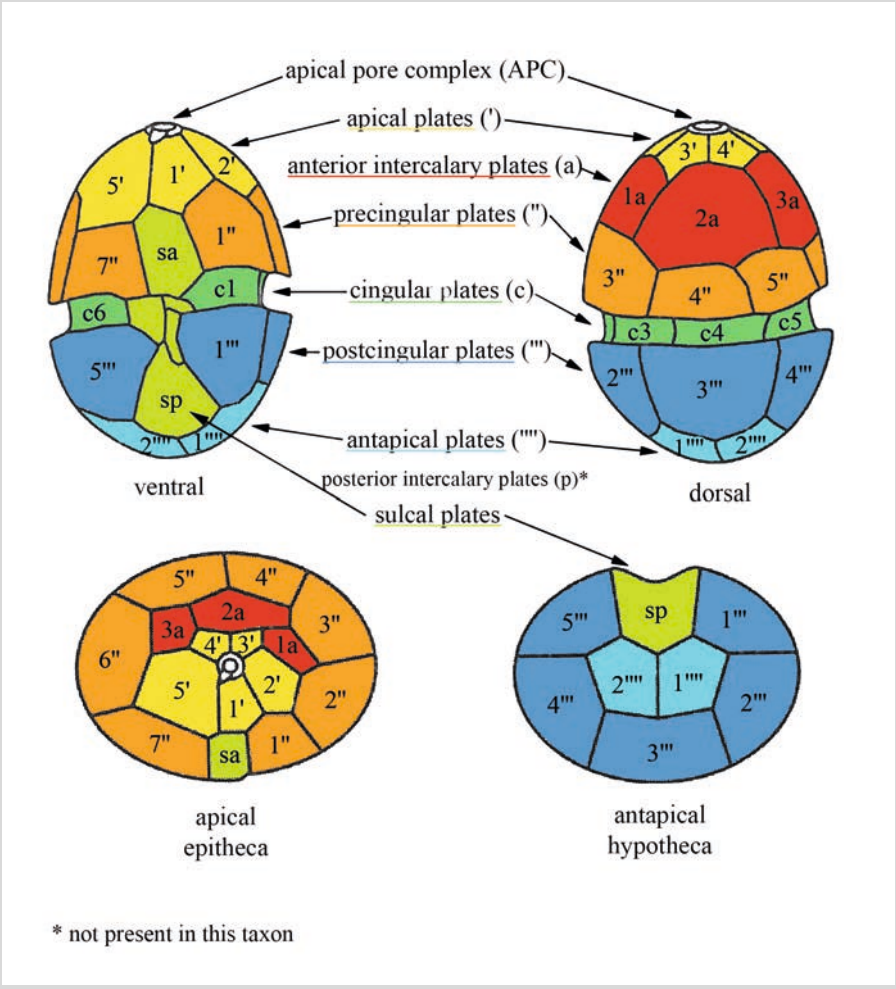


Fig. 2: Kofoid system of thecal plate designation.

out change of the author citation or date (see also Art. 60.12)." For the holotype designation in many published (past) new dinoflagellate species descriptions article 40.5 applied and still applies: "For the purpose of Art. 40, the type of a name of a new species or infraspecific taxon of microscopic algae or microfungi (fossils excepted: see Art. 8.5) may be an effectively published illustration if there are technical difficulties of preservation or if it is impossible to preserve a specimen that would show the features attributed to the taxon by the author of the name."

II. 'Materials & Methods'

Habitats

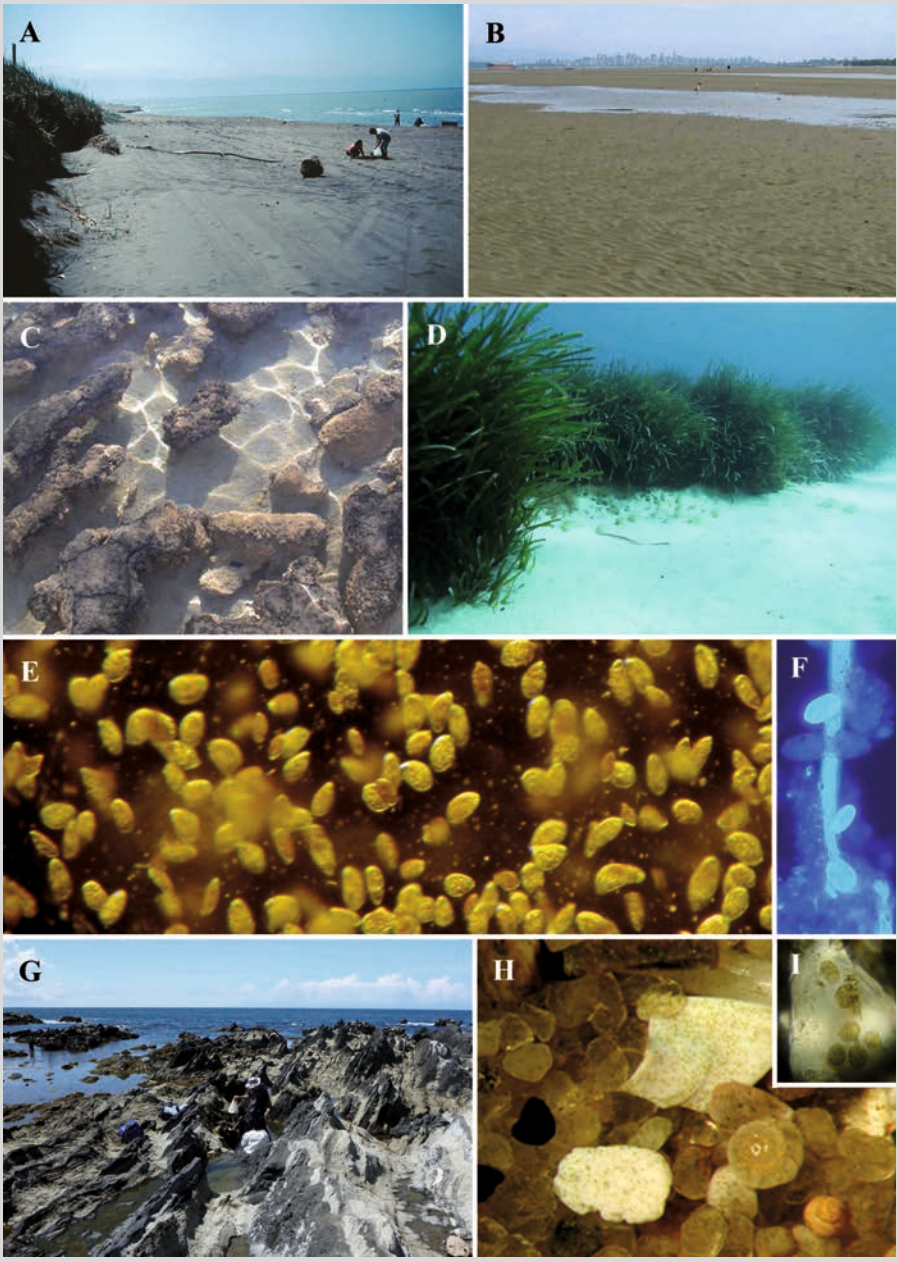
Benthic dinoflagellates inhabit sediments of beaches, intertidal flats, subtidal areas, tide pools, are epiphytic on seaweeds and seagrass, attached to coral, or rarely, are epilithic (Fig. 3). In sediments they prefer to live in the interstitial spaces of sand of medium grain size, but they can also occur in coarser or finer sand and in coral rubble. Some may occur on the surface of mud flats, but in this habitat dinoflagellates are more frequently found in water holes, for example crab burrows or sediment cracks.

Sampling

Intertidal or shallow subtidal sediments are collected with a spoon or collecting/sampling tube (e.g. Hoppenrath 2000b, Murray 2003) into a plastic container (Fig. 4). The upper 0.5 to 20 cm of the sediment are generally taken. It is recommended to sample at least the upper 5 cm of sandy sediments. When sampling in the supralittoral zone of beach habitats, it is usually necessary to dig into the sediment to the point where the seawater begins to seep into the hole (e.g. Horiguchi and Kubo 1997). The seepage is then collected in plastic bottles. Intertidal flats are usually sampled during low tide. Sublittoral samples are taken by divers (snorkeling or scuba diving) or with a sediment box corer from a research vessel (e.g. Hansen et al. 2001, Hoppenrath 2000b, e) or are collected with artificial surfaces (plastic screens) suspended in the water (e.g. Faust 1995).

Seaweeds and seagrasses are detached from the sediment or rocks and placed in plastic bags or bottles (e.g. Kohli et al. 2013, Okolodkov et al. 2007). This is followed by weighing (usually measurements are taken as

→ Fig. 3: Habitats. A: Sandy beach, Ishikari Beach, Japan. B: Sandy intertidal flat, English Bay, Vancouver, Canada. C: Sandy sediment between stromatolides, Shark Bay, Australia. D: Seagrass meadow, Elba, Italy; photo courtesy of HYDRA Institut für Meereswissenschaften. E: Epiphytic cells of *Ostreopsis siamensis* on *Padina*; photo courtesy of N. L. Nguyen. F: Epiphytic cells of *Prorocentrum rhathymum* on a macroalga, epifluorescence image of stained material; photo courtesy of T.V. Ho. G: Tide pools from lower to higher intertidal area, Arasaki Beach, Japan. H: Coarse beach sediment with epilithic dinoflagellate cells; photo courtesy of P. Houpt. I: Detail showing the epilithic *Spiniferodinium* cells; photo courtesy of P. Houpt.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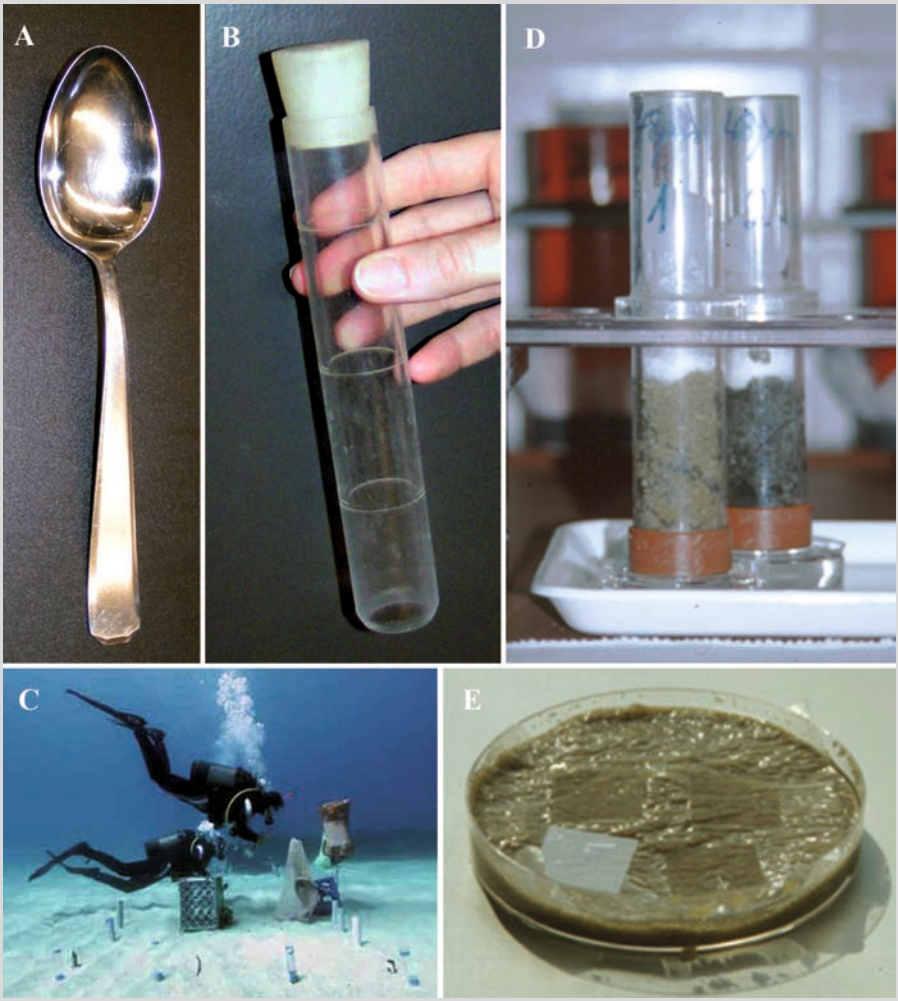

Fig. 4: Sampling and extraction. A: A simple spoon for sampling at low tide in intertidal flats. B: A sampling tube for sampling at low tide or below the water surface. C: Scuba diving in sublittoral areas; photo courtesy of HYDRA Institut für Meereswissenschaften. D: "Uhlig method", extraction with melting seawater ice. E: "Coverslip method".

wet weight rather than dry weight) and identifying the species of macroalgae or seagrass and storing at the seawater temperature until extraction. As many epiphytic dinoflagellates can also inhabit the water column directly surrounding the surface of macroalgae, a plankton net (approximately 20 µm mesh) can be pulled over the top of a shallow seagrass bed or area of dense macroalgal growth (in shallow subtidal habitats), from a jetty or pier, or using a small boat. This sampling method is not quantitative, but can provide dense samples for culturing or identification of species. In shallow tide pools, both water column and sediment samples can be taken. Samples can be used to extract living cells or were fixed before cell isolation.

Extraction = separation from the substrate

Dinoflagellates can be separated from the sand by extraction with seawater ice through a fine filter (the "Uhlig method" described in detail in Uhlig 1964, Hoppenrath 2000b). Living cells accumulate in a Petri dish beneath the filter (extraction tube) (Fig. 4) and can be observed with an inverted light microscope. Alternatively, sediment can be placed in trays, covered with a layer of tissue and coverslips (Webb 1956, Hoppenrath 2000b, Murray 2003) (Fig. 4). Coverslips are removed after several hours and living cells attached to the coverslips can be directly investigated with a light microscope (LM). Both methods have different selectivity for species, and not all species will be extracted by both/these methods (Hoppenrath 2000b). The "Uhlig-method" mainly extracts free-swimming motile cells and the "Webb- or coverslip-method" often selects species gliding on or attaching to surfaces. The "Uhlig-method" only works with sandy sediments, whereas the coverslip-method will work for all kinds of sediment, including very fine sediments. Combining different extraction methods is therefore recommended to detect the most complete community.

A different approach that has been used is suspending sediment with filtered seawater, mixing thoroughly, and then filtering through fine gauze in two steps (150 and 80 µm), finally concentrating by filtration through 20 µm gauze (e.g. Selina and Hoppenrath 2013), resulting in a 20–80 µm fraction containing the living cells.

For epiphytic species, in general the separation method involves vigorous shaking, sonicating or scrubbing of the seaweeds or seagrass in seawater (e.g. Aligizaki et al. 2009, Hansen et al. 2001, Litaker et al. 2009, Mohammad-Noor et al. 2007b, Okolodkov et al. 2007). The suspension can be then sieved through a filter series or filtered once to get a concentrated sample.

Sampling with artificial surfaces (plastic screens) suspended in the water close to the bottom (e.g. Faust 1995, Kibler et al. 2010 BHAB workshop handout) also extracts cells from the environment. During "suction sampling" a suspension (produced by substarte agitation) will be collected with a syringe or small solid benthic surfaces (e.g. dead coral or rocks) will be sampled with a vacuum hose connected to a bottle and vacuum pump (Kibler et al. 2010 BHAB workshop handout).

Fixation and Electron Microscopy (EM)

Raw samples or extracted dinoflagellates can be fixed with glutaraldehyde, formalin or Lugol's solution. Manually isolated or cultured

clean dinoflagellate cells can be fixed and prepared in various procedures for transmission (TEM) and scanning electron microscopy (SEM).

For TEM, the most important step is the choice of chemicals and conditions for the first fixation. The best fixation conditions can be different from species to species and therefore, a suitable fixation method for each species must be developed by modifying published protocols (e.g. Horiguchi and Pienaar 1988a, Horiguchi et al. 2011, Pienaar et al. 2007). Instead of chemical fixation, the high pressure freezing method can obtain good results (see for example, Yamada et al. 2013).

One method is to make use of natural samples for TEM sectioning. This method is also applicable to the species which are difficult to culture, such as heterotrophic dinoflagellates. For example, the samples collected from the surface of seaweeds usually contain multiple species of benthic dinoflagellates. The seawater containing dinoflagellates is fixed, dehydrated and embedded in resin as usual, then the resin containing dinoflagellate cells is spread over the piece of Overhead projector (OHP) sheet (Note: OHP sheets for inkjet printer are not suitable for this purpose), and is sandwiched with another piece of OHP sheet and this is polymerized in the oven. After polymerization, one side of OHP sheet should be removed. The resultant thin embedded sample is easy to observe under the microscope and the target cells can be easily spotted and marked by a marker pen. A small piece of resin (ca. 3 mm x 3 mm) containing target cells is cut out by razor blade and the piece is stuck onto the tip of a sample block with instant glue. Then, the sample can be trimmed, sectioned and observed as usual. Another useful technique is the 'single cell TEM method'. This method can be applicable to very 'rare', non-culturable species (Onuma and Horiguchi 2013).

Although most modern SEMs are equipped with an environmental mode (E-SEM) allowing to work at low vacuum, and with some hydrated and uncoated specimens, we have not been able to obtain good results and pictures of benthic dinoflagellates detailed enough for taxonomic identification. Hence, we prefer using normal SEM with full vacuum, but for this reason, a process with several steps is necessary to dehydrate samples without the collapse of the cell membranes or thecae (Couté 2002). Fixation is a critical step and several fixatives (formaldehyde, glutaraldehyde, osmium tetroxide) are commonly used. Cells must be transferred from seawater to absolute ethanol through several steps of increasing ethanol concentrations. The last step of dehydration can be either CO_2 critical-point drying or a chemical alternative like hexamethyldisilazane (HMDS). Alternatively, a recently developed method successfully used tert-butanol in place of the ethanol series (Won Jung et al. 2010). Chomérat and Couté (2008) used with some success a special clamp-device that traps cells within it. The whole assembly is processed for dehydration and critical-point dried. It must be kept in mind that because of the numerous steps and transfers of the specimens, the most critical aspect of the preparation is the loss of material during the process. Takano and Horiguchi (2006) described a method that they used to successfully obtain light microscope, SEM and molecular genetic data from the same single cell.

To prepare the unarmoured dinoflagellates for SEM, isolated cells are allowed to settle on a poly-l-lysine-coated glass plate and fixed by the vapor of 4% osmium tetroxide for several seconds. The plate with fixed cells is dehydrated, critically point dried, sputter coated

and observed as usual (e.g. Takano and Horiguchi 2006). The fixation can also be done by 1% aqueous osmium tetroxide, but in this case, the cells should be rinsed by distilled water before dehydration.

Culturing

Extracted living cells can be isolated (micropipetting, dilution) and cultured with diverse approaches and media (e.g. f/2, K, ES-DK) and publications dealing with phototrophic species should be consulted (e.g. Litaker et al. 2009). Only one heterotrophic benthic species has been cultured to date, and was fed on small cryptophytes (Larsen 1988). Generally, many species are adapted to lower light conditions and grow only slowly. For some species it will be of advantage to not place the isolated and washed cell(s) directly in medium but first in sterile seawater of the sampling locality, then adding a small amount of medium and slowly increasing the medium proportion step by step during the culture starts to grow. This procedure allows gradual acclimation of the specimens to the medium. Some taxa will start to grow more easily in culture when in company with other cells.

Quantification

Fixed cells can be counted in standard counting or settling chambers. Counts can be given as cells/g seaweed or seagrass wet weight, cells/cm^3 or ml sediment or water, cells/cm^2 or m^2 sediment or artificial surface area.

The "epiphyte method" of quantification involves simply shaking the cells free from the macroalgae, fixation, and then recording the quantities (cell counts in a chamber) as cells/g wet weight algae (e.g. Aligizaki et al. 2009, Mangialajo et al. 2008, Okolodkov et al. 2007), see Extraction above and Ecology chapter below. The "artificial surface method" is principally the same but cell counts can be related to the surface area.

For the extraction and enumeration of flagellates (including dinoflagellates) from sandy sediment samples, a modified "decant/fix method" was described by Lee and Patterson (2002a), involving sonicating the sediment in a fixative solution. The estimated abundances and biomasses were probably underestimated by this method (Lee and Patterson 2002b).

Hoppenrath (2000b) quantified living species extracted from sandy sediments with the seawater ice method (see above). Sediment samples of known volume were taken with a collecting tube. The sediment was extracted two or three times (depending on the expected cell densities) and living cells were counted directly after extraction in the Petri dishes with an inverted microscope. The complete dish was screened. Samples were not fixed to be able to count the naked species. This is not a standardised method, and it is likely that the error involved in the quantification of every species would likely differ, as they each had different behaviour, and this was estimated (Hoppenrath et al. 2000b). Moreover, the extraction efficiency of the seawater ice method differs depending on the species.

For *Ostreopsis* a quantitative real-time PCR method (qrt-PCR) to enumerate species in environmental samples has been developed (Perini et al. 2011). Vandersea et al. (2012) developed a species-specific semi-quantitative polymerase chain reaction assay (qPCR) for *Gambierdiscus*. Both approaches were based on the SYBR green technique. For *Gambierdiscus* a detection limit for ten cells per sample was recorded (Vandersea et al. 2012).

III. Taxonomy

Adenoides [Aden: gland; eidos: sight – neutral]

***Adenoides* Balech**

Publication: Balech, 1956, Revue Algologique 2, pp. 30–31, Figs 1–8.

Type species: *A. eludens* (Herdman) Balech.

Plate formula: APC 4′ 6c 4s 5‴ 5p 1⁗⁗ or APC 4′ 6c 5s 5‴ 3p 2⁗⁗.

Description: Thecate genus with laterally flattened cells with a minute, depressed and scarcely visible epitheca. Shallow cingulum without displacement almost at the anterior cell end. No precingular plate series.

Remarks: A taxonomic problem with the original description of the type species has been discussed in detail in Hoppenrath et al. (2003, pp. 385, 389) who reinvestigated and revised the description of *A. eludens*. Whether a second *Adenoides* species, described by Herdman (1922) as *Amphidinium* species and transferred to *Adenoides* by Dodge (1982; without own observations), really exists, is not clear. Because of this uncertainty it has not been included herein.

***Adenoides eludens* (Herdman) Balech**

Publication: Balech, 1956, Revue Algologique 2, p. 30.

Basionym: *Amphidinium eludens* E.C. Herdman; Herdman 1922, Proceedings and Transactions of the Liverpool Biological Society 36, pp. 22–23 (26), Figs 1, (2).

Illustrations: Figs 5, 6.

Size: 25–40 µm long, 22–28 µm deep.

Plate formula: APC 4′ 6c 4s 5‴ 5p 1⁗⁗ or APC 4′ 6c 5s 5‴ 3p 2⁗⁗.

Chloroplasts: Two lobed brown peridinin-chloroplasts.

Description: Round to oval, asymmetrical, laterally flattened cells with minute depressed and scarcely visible epitheca. The hypotheca is longer dorsally than ventrally. Smooth thecal plates with pores. Shallow cingulum without displacement almost at the anterior cell end. Short and slightly depressed sulcus with one flagellar pore located in the anterior third of the cell. No precingular plate series. Two conspicuous large pores at the dorsal posterior end. Two striking pyrenoids visible as rings because of the starch sheaths. Nucleus in the lower dorsal hyposome half.

Distribution: Sandy sediments. Port Erin, Isle of Man, UK (Herdman 1922); North Sutherland, Scotland, UK (Dodge 1989); North German Wadden Sea, Germany (Hoppenrath 2000b, Hoppenrath et al. 2003); Normandy, France (Paulmier 1992); Roscoff, Brittany, France (Balech 1956, Dodge and Lewis 1986); Elba, Italy (Hoppenrath unpubl. obs.); Arabian Gulf, Kuwait (Saburova et al. 2009, Al-Yamani and Saburova 2010); Sea of Japan, Russia (Konovalova and Se-

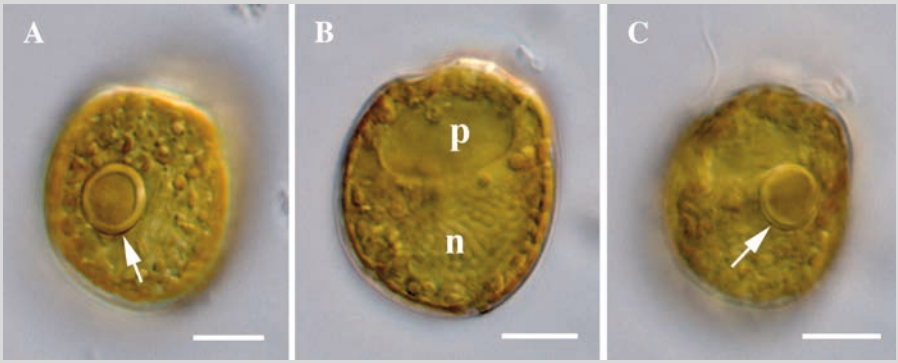

Fig. 5: *Adenoides eludens*. A–C: Different focal planes, note the ring-like starch sheath around the pyrenoid (arrow); p = pusule, n = nucleus. Scale bars: 10 µm.

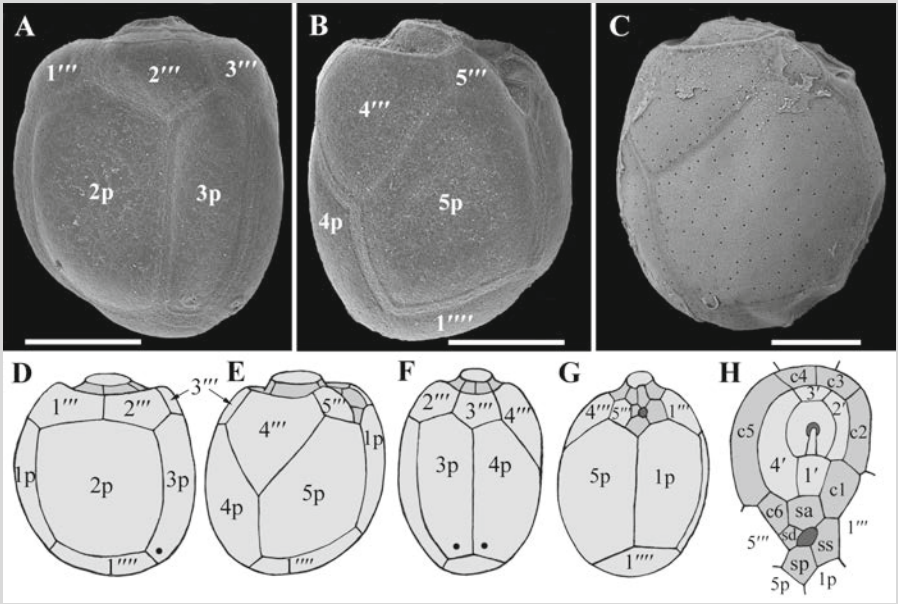

Fig. 6: *Adenoides eludens*. A: Left lateral view. B, C: Right lateral view; note the thecal pores in C. D–H: Drawings of the plate pattern. D: Left lateral. E: Right lateral. F: Dorsal. G: Ventral. H: Epitheca, cingulum and sulcus. Scale bars: 10 µm.

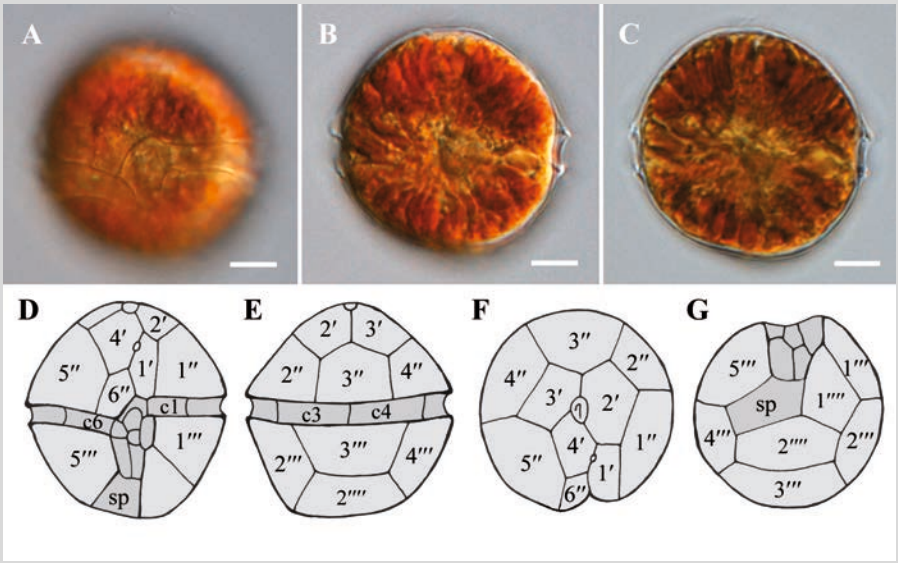

Fig. 7: *Alexandrium hiranoi*. A–C: Different focal planes; scale bars: 10 µm. D–G: Drawings of the plate pattern, modified after Kita and Fukuyo (1988). D: Ventral. E: Dorsal. F: Apical, epitheca. G: Antapical, hypotheca and sulcus.

lina 2010); Izu Peninsula, Shizuoka, Japan (Hara and Horiguchi 1982); Boundary Bay, BC, Canada (Baillie 1971, Hoppenrath unpubl. obs.).

References: Dodge (1982), Hoppenrath et al. (2003), Lebour (1925), Schiller (1933), Steidinger and Tangen (1997).

Alexandrium [from Alexandria, type locality – neutral]

***Alexandrium* Halim**

Publication: Halim, 1960, Vie Milieu 11, p. 102, Figs 1a–d.

Type species: *A. minutum* Halim.

Plate formula: APC 4′ 6″ 6c (8)9–10s 5‴ 2⁗.

Description: Gonyaulacoid genus with excavated, descending cingulum without overhang and without or only narrow lists. Dino-chloroplasts present. Transversely elongated nucleus. Morphological characters important for species identification are cell size and shape, thecal ornamentation, cingular and sulcal excavation, sulcal lists and shape of some sulcal plates (sa, ssa, sp), shapes of the APC, first apical and sixth precingular plates, chain formation ability (e.g. Balech 1995).

Remarks: Planktonic genus with so far 31 described species. For species identification specimens need to be stained or dissected

or investigated by SEM. For the morphological details of the species see the monograph from Balech (1995) and for general information the review by Anderson et al. (2012).

***Alexandrium hiranoi* Kita et Fukuyo**

Publication: Kita and Fukuyo, 1988, Bulletin of Plankton Society of Japan 35, p. 2 and 4, Fig. 1.

Synonyms: *Goniodoma pseudogonyaulax* sensu Silva (1965), Kita et al. (1985), non Biecheler (1952); *Alexandrium pseudogonyaulax* sensu Horiguchi (1983).

Illustrations: Fig. 7.

Size: 18–75 µm long, 18–75 µm wide.

Plate formula: APC 4′ 6″ 6c 8s 5‴ 2⁗.

Chloroplasts: Brown peridinin chloroplasts.

Description: Round cells, sometimes longer than wide with moderate flattened ant-apex and smooth thecal plates. Shallow sulcus and excavated cingulum descending about one cingulum width. Narrow oval Po with nearly parallel sides. Pentagonal and narrow plate 1′ with ventral pore. C-shaped nucleus in the equatorial plane.

Similar species: *A. pseudogonyaulax,* but *A. hiranoi* with longer epitheca and differently shaped first apical and sulcal plates (Balech 1995).

Remarks: The species is forming dense blooms and has a bentho-pelagic life cycle, dividing in the benthic vegetative (temporary) cyst stage (Kita et al. 1985). Sexual reproduction and a benthic resting cyst stage are also known (Kita et al. 1993). *Alexandrium hiranoi* can produce goniodomins that cause paralysis and mortality in finfish.

Distribution: Tidal pools. Obidos lagoon, Spain (Silva 1965); Jogashima Island and Arasaki, Kanagawa, Japan (Kita and Fukuyo 1988).

References: Anderson et al. (2012), Balech (1995), Kita et al. (1985, 1993).

Amphidiniella [Amphidinium; diminutive suffix -ella – feminine]

***Amphidiniella* Horiguchi**

Publication: Horiguchi, 1995, Phycological Research 43, p. 93.

Type species: *A. sedentaria* Horiguchi.

Plate formula: Po 4′ 1a 7″ 5c 4s 6‴ 2⁗.

Description: Thecate genus with dorsoventrally flattened cells having a small epitheca and a large hypotheca, containing a chloroplast.

***Amphidiniella sedentaria* Horiguchi**

Publication: Horiguchi, 1995, Phycological Research 43, pp. 93–94, Figs 1–20.

Illustrations: Figs 8, 9.

Size: 14–20 µm long, 10–15 µm wide, 6–7 µm deep.

Plate formula: Po 4′ 1a 7″ 5c 4s 6‴ 2⁗.

Chloroplasts: One typical yellow-brown peridinin chloroplast with pyrenoid with starch sheath.

Description: Oval to ovoid dorsoventrally flattened cells with small fan-shaped (ventral view) or cap-like (dorsal view) asymmetrical epitheca (about one third of the cell length) and sack-shaped hypotheca. The epitheca has ventrally a posterior triangular fringe. Small notches at the anterior cell end. The ascending cingulum (about one cingular width) completely encircles the cell. The sulcus widens towards the posterior of the cell. Pyrenoid with starch-sheath in

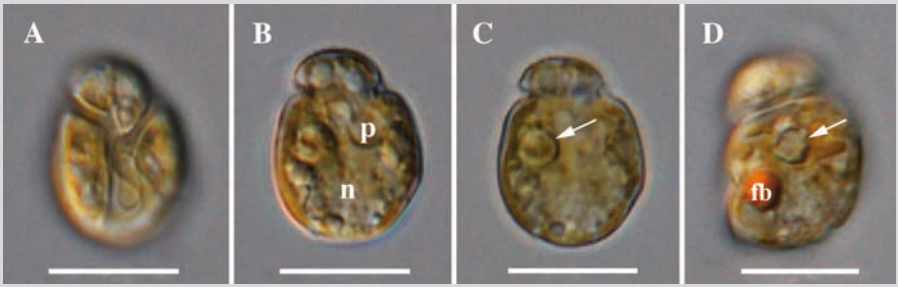

Fig. 8: *Amphidiniella sedentaria*. A: Ventral view. B: Mid cell focus showing the pusule (p) and the nucleus (n). C: Note the starch ring around the pyrenoid (arrow). D: Note the pyrenoid (arrow) and food body (fb). Scale bars: 10 µm.

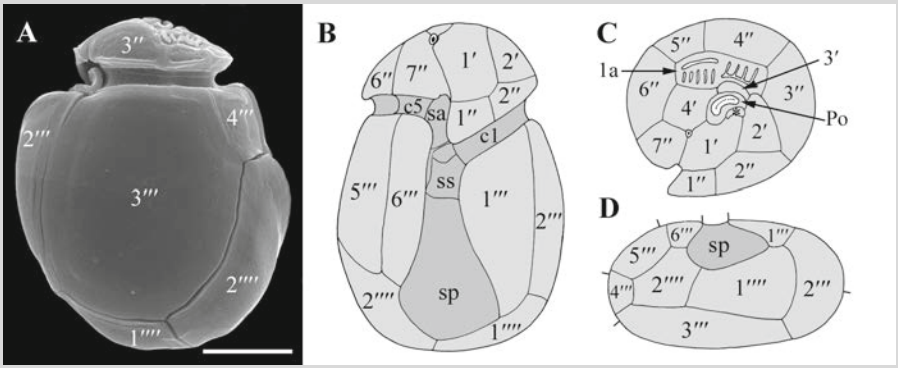

Fig. 9: *Amphidiniella sedentaria*. A: Dorsal view, SEM (photo by N. Borchhardt), scale bar: 5 µm. B–D: Drawings of the plate pattern, modified after Horiguchi (1995). B: Ventral. C: Apical, epitheca. D: Antapical, hypotheca and part of the sulcus.

the middle of the right cell half. Nucleus in the posterior part of the cell. Smooth thecal plates with pores, except for the first apical intercalary plate (1a) with strong ornamentation. Relatively large bean-shaped apical pore plate with slit-like apical pore covered by a lip-shaped projection. Ventral pore adjacent to the first apical plate (1′) and in contact with plates 4′ and 7″.

Similar species: Murray (2003) recorded a similar species with apical hook under the name *Amphidiniella* sp 1. This taxon was also discovered in Germany, France, Italy and Kuwait (Hoppenrath, Chomérat and Saburova unpubl. data) and it seems not to be related to *Amphidiniella* (new genus description in preparation).

Remarks: In culture the species attaches,

living mainly motionless as film but also can swim quite rapidly (Horiguchi 1995).

Distribution: Sandy beaches, intertidal sand flats or on the surface of dead coral. Elba, Italy (Borchhardt and Hoppenrath unpubl. obs.); Palm Beach, Kwazulu-Natal, South Africa (Horiguchi 1995); Shark Bay, Australia (Al-Qassab et al. 2002); Sesoko Beach and Ondo, Okinawa, Japan (Horiguchi 1995, Horiguchi unpubl. obs.); Sydney, Australia (Murray 2003).

References: Al-Qassab et al. (2002), Murray (2003).

Amphidiniopsis [Amphidinium; opsis: aspect – feminine]

***Amphidiniopsis* Wołoszyńska**

Publication: Wołoszyńska, 1928, Archives d'Hydrobiologie et d'Ichtyologie 3, p. 256.

Type species: *A. kofoidii* Wołoszyńska.

Plate formula: 3' 7'' 5''' 2'''' (original description); APC 3–4' 1–3a 6–8'' 3–8c 3–5s 5''' 2'''' (today).

Description: Thecate genus with cells having a smaller epitheca and a large hypotheca. Currently the genus is characterized by an ascending cingulum, a distinctive curved sulcus and hypothecal plate pattern (Hoppenrath et al. 2009b). Species are laterally or dorsoventrally flattened, with a complete or incomplete cingulum, and with or without an apical hook. Diverse cell morphologies have been described and morphological variability is known (e.g. Selina and Hoppenrath 2008). Three major subgroups can be recognized: (1) laterally flattened species with complete cingulum, (2) dorsoventrally flattened species with complete cingulum, sulcus positioned in the middle of the cell, no apical hook, and one or two anterior intercalary plates, and (3) dorsoventrally flattened species with complete or incomplete cingulum, sulcus positioned in the middle of the cell (the deepened part of the sulcus can be shifted to the left side), with an apical hook pointing to the left, and three anterior intercalary plates (Hoppenrath et al. 2012b).

Group 1: *A. arenaria, A. dentata, A. galericulata, A. kofoidii, A. sibbaldii*.

Group 2: *A. aculeata, A. hexagona, A. hirsuta, A. konovalovae, A. striata, A. swedmarkii* (*A. rotundata* but with shifted sulcus and three anterior intercalary plates?).

Group 3: *A. korewalensis, A. pectinaria, A. uroensis* (*A. cristata* but with only one anterior intercalary plate? *A. dragescoi* and *A. rotundata* but without apical hook?). All currently known species are heterotrophic, benthic (sand-dwelling) and marine, except of one freshwater species (*A. sibbaldii*).

Remarks: The history of records, nomenclatural changes and classification schemes of the species were summarized in Hoppenrath (2000f) and Hoppenrath et al. (2009b). A revision of the genus is needed and it is possible that it contains several subgenera or that it is polyphyletic (e.g. Hoppenrath et al. 2012b). *Herdmania* seems to be closely related to *Amphidiniopsis* (Yamaguchi et al. 2011a, Hoppenrath et al. 2012b).

***Amphidiniopsis aculeata* Hoppenrath, Koeman et Leander**

Publication: Hoppenrath et al., 2009b, Marine Biodiversity 39, pp. 4–6, Figs 1–2, 3A.

Illustrations: Figs 11A–C.

Size: 38–40 µm long, 32–35 µm wide.

Plate formula: APC 4' 2a 7'' 3c 5s 5''' 2''''.

Chloroplasts: none.