rial suitable for dating such events. We have reported one such peat bed within the sand horizons of the western coastal sedimentary sequence of North Andaman[1].

1. Ray, S. K. and Acharyya, A., 26 December 2004 earthquakes: coseismic vertical ground movement in the Andaman Islands. *Geol. Surv. India Spec. Publ.*, 2007, **89**, 63–81.
2. Curray, J. R., Tectonics and history of the Andaman sea region. *J. Asian Earth Sci.*, 2005, **25**, 187–232.
3. Pal, T., Chakraborty, P. P., Gupta, T. D. and Singh, C. D., Geodynamic evolution of the outer arc–forearc belt of the Andaman Island, the central part of the Burmese–Java subduction complex. *Geol. Mag*., 2003, **140**, 289–307.
4. Karunakaran, C. *et al.*, Geology of Great Nicobar Island. *J. Geol. Soc. India*, 1975, **16**, 135–192.
5. Bilham, R., A flying start, then a slow slip. *Science*, 2005, **308**, 1126–1127.
6. Lay, T. *et al.*, The Great Sumatra–Andaman earthquake of 26 December 2004. *Science*, 2005, **308**, 1127–1133.
7. Stein, S. and Okal, A., Speed and size of the Sumatra earthquake. *Nature*, 2005, **434**, 581–582.
8. Plafker, G. and Kachadoorian, R., Geologic effects of the March 1964 earthquake and associated seismic sea waves on Kodiac and nearby islands, Alaska. *US Geol. Surv. Prof. Pap.*, 1966, **543-D**, D1–D46.
9. Plafker, G. and Savage, J. C., Mechanism of Chilean earthquake of May 21 and 22, 1960. *Bull. Geol. Soc. Am*., 1970, **81**, 1001–1030.
10. Carver, G. A., Jayke, A. S., Valentine, D. W. and Li, W. H., Coastal uplift associated with the 1992 Cape Mendocino earthquake, northern California. *Geology*, 1994, **22**, 195–198.
11. Carver, G. A. and McCalpin, J. P., Paleoseismology of compressional tectonic environments. In *Paleoseismology* (ed. McCalpin, J. P.), Academic Press, London, 1996, pp. 183–270.
12. Meltzner, A. J. *et al.*, Uplift and subsidence associated with the great Ache–Andaman earthquake of 2004. *J. Geophys. Res.*, 2006, **111**, B02407.
13. Rajendran, C. P. *et al*., Crustal deformation and seismic history associated with the 2004 Indian Ocean earthquake: A perspective from the Andaman–Nicobar islands. *Bull. Seismol. Soc. Am.*, 2007, **97**(1A), S174–S191.
14. Schumm, S. A., Dumont, J. E. and Holbrook, J. L., *Active Tectonics and Alluvial Rivers*, Cambridge University Press, Cambridge, UK, 2002, p. 276.
15. McCalpin, J. P. and Nelson, A. R., Introduction to paleoseismology. In *Paleoseismology* (ed. McCalpin, J. P.), Academic Press, London, 1996, pp. 1–32.
16. Bodin, P. and Klinger, T., Coastal uplift and mortality of intertidal organisms caused by the September, 1985 Mexico earthquake. *Science*, 1986, **233**, 1071–1073.
17. Paul, J. *et al*., Postseismic deformation of the Andaman Islands following the 26 December, 2006 great Sumatra–Andaman earthquake. *Geophys. Res. Lett.*, 2007, **34**, L19309.
18. Kar, R. and Kar, R. K., Mangroves can check the wrath of tsunami. *Curr. Sci.*, 2005, **88**, 675.

ACKNOWLEDGEMENTS. A research grant to S.K.R. from the Department of Science and Technology, New Delhi helped pursue this research work that was started in 2005 at the Geological Survey of India (GSI), Kolkata. A.A. participated in the May 2005 fieldwork that was supported by the GSI. Sandip Neogi helped with the preparation of the figures.

Received 29 November 2010; revised accepted 31 May 2011

Sexual reproduction in *Odontella regia* (Schultze) Simonsen 1974 (Bacillariophyta)

S. Hegde, D. D. Narale and A. C. Anil*

National Institute of Oceanography (CSIR), Dona Paula, Goa 403 004, India

We report here on the sequence of spermatogenesis and sperm cell count of *Odontella regia* (Schultze) Simonsen from Indian waters. The sequence of events in the spermatogenesis producing 16 spermatogonia following four differentiating (depauperating) mitosis to produce 64 sperms per cell and dehiscence is reported. Fertilization, auxosporulation and size restoration in *O. regia* are also shown.

Keywords: Depauperating mitosis, diatom, *Odontella regia*, sexual reproduction, spermatogenesis.

THE most characteristic feature of a diatom is the bipartite (made up of two halves), silicious cell wall, called the frustule. One half is larger (epitheca) and overlaps the other half, which is slightly smaller (hypotheca), analogous to a box. Diatoms reproduce vegetatively by binary fission, and two new individuals are formed within the parent cell using the parent theca as the epitheca and producing a new hypotheca. Therefore, one daughter cell retains the original size, whereas the other is slightly smaller. This brings about a steady reduction in size with every mitotic or vegetative division, and was described by the MacDonald–Pfitzer rule[1,2]. A gradual decrease in the cell diameter in centric diatoms and cell length in pennate diatoms is usually observed. Generally a diatom cell undergoes gametogenesis when it reaches about 30–40% of it original size. This size is believed to be the cue for the 'biological clock'[3], to which the diatoms respond with sexual reproduction to restore cell size. Restoration of cell size by sexual reproduction is therefore a unique feature of the diatom community[4], making sexuality obligatory rather than a factor in dormancy or dispersal[5].

It is well known that diatoms are sexual organisms. However, sexual stages are known from only a tiny minority of diatom species[6]. This lack of observation has been attributed to the long time intervals between each sexual phase in a population[5,7]. Furthermore, only a small proportion of a vegetative population is involved[8–10], thereby increasing the chances of missing out witnessing sexual reproduction.

Although 'auxosporulation' was mentioned in 1847 (ref. 11) and 'microspores' were reported as early as 1927 (ref. 12), the fact that centric diatoms underwent oogamy was only established in 1950 (ref. 13). Most of the earlier

*For correspondence. (e-mail: acanil@nio.org)

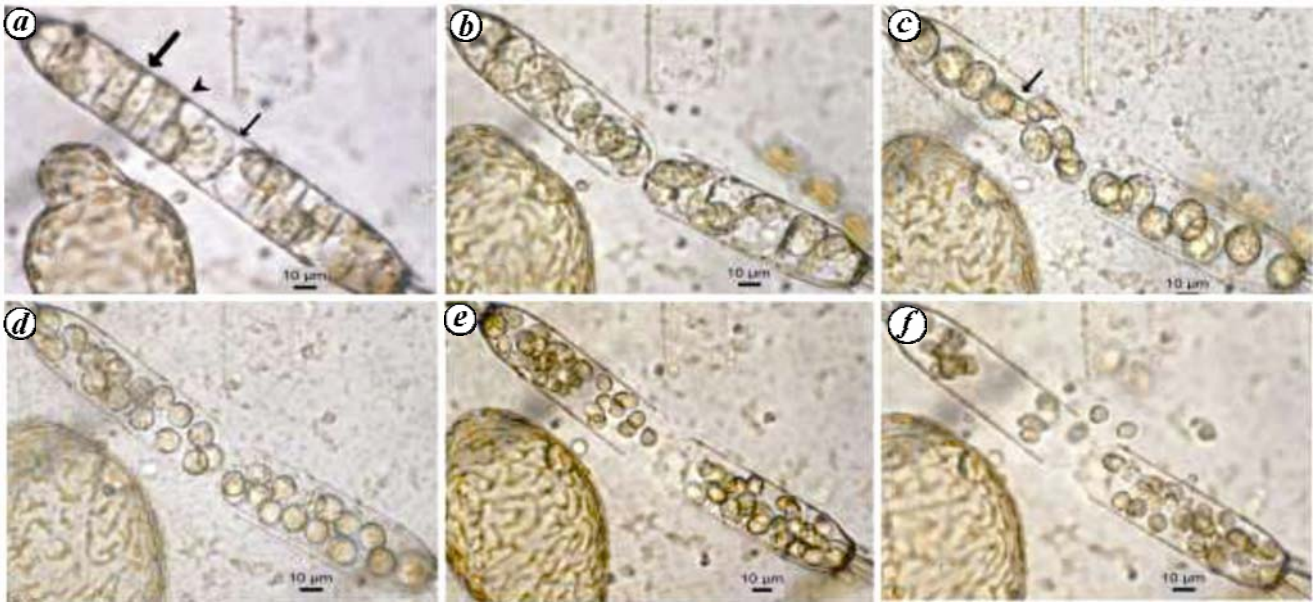

Figure 1. *Odontella regia*. ***a***–***f***, Spermatogenesis. ***a***, Spermatogonangium has undergone the first special differentiating mitosis with the formation of the rudimentary theca (arrow) which is placed immediately after the first mitotic division forming two half-spermatogonangia. The upper half-spermatogonangium has completed the second mitosis (thick arrow) and progresses to the third (arrowhead), whereas in the lower half-spermatogonangium the second mitosis is not yet complete but the third has begun, indicating that it occurs in quick succession. ***b***, The upper half-spermatogonangium is at the fourth differentiating mitosis with the formation of eight spermatogonia (16 spermatogonia are produced in this stage, however, the two half-spermatogonangia were asynchronous). ***c***–***e***, These special mitoses are then followed by two meiotic divisions, wherein each spermatogonium divides into four flagellated sperms giving rise to 32 sperm cells in each half-spermatogonangium. ***c***, Meiotic division progresses (arrow) to produce 16 cells in the upper spermatogonangium, whereas the lower one has just completed forming eight cells. ***d***, The upper half-spermatogonangium has 16 cells and the lower one is still dividing to produce 16 cells. ***e***, The 64-cell stage; 32 cells in each half-spermatogonangium, and ***f***, Dehiscence. The upper spermatogonangium has released the 32 sperm cells produced, whereas the lower one is not ready for dehiscence. (Also note the rounding-off of the oogonium on the left in ***a***–***f***.)

works on sexual reproduction were non-English publications and Chepurnov *et al.*[6] point this out as the other possible reason that sexual reproduction in diatoms is not well known. Studies on the sexual reproduction in centric diatom species are detailed in the literature[4–7,9,10,14–16]. In India, however, there has been scarcely any work on the life histories of Indian diatoms since the studies by Subrahmanyan[17,18], Rao and Desikachary[19], and Ragothaman and Rao[20].

We describe here details of sexual cycle of *Odontella regia* (Schultze) Simonsen 1974 (synonym *Biddulphia regia* (Schultze) Ostenfeld). The vegetative cycle has been described by Mayer and Schmid[21].

O. regia is a planktonic, bipolar, centric diatom in the Eupodiscaceae family, with a probable cosmopolitan distribution[22]. Cells are narrow elongated, apical length is 137.4 µm and trans-apical length 37.6 µm. Trans-apical length increased to 115 µm and apical length decreased to 40 µm following sexual reproduction.

O. regia has been shown to auxosporulate[14], but details of this process and the number of sperm cells produced was not mentioned. We have recorded 64 sperm cells per cell in this species.

Sea-water samples were collected on 2 May 2010 from Dona Paula Bay located at the mouth of the Zuari estuary in Goa (15°27.5′N, 73°48′E), on the west coast of India for isolation of algal clones. A subsample was filtered through 100 µm mesh to exclude herbivores and then concentrated up to ten times on a 10 µm mesh. Subsequently, serial dilution from 10^{-1} to 10^{-5} was carried out in a six-well polystyrene culture plate in 10 ml of f/2 (ref. 23) enriched medium. Incubation was carried out at 24°C under illumination from a fluorescent lamp (50 µmol photons m^{-2} s^{-1}) in 12 : 12 light and dark photocycle. Gametogenesis in *O. regia* was observed in culture wells following 3 days of incubation. Olympus IX71 (Olympus Singapore Pvt Ltd, Singapore) inverted microscope equipped with a digital camera (Olympus CAMEDIA C-4040ZOOM) was used. For nuclear staining, the culture was fixed with 1% glutaraldehyde and stained with DAPI (4-6,diamidino-2-phenylindole; Sigma, USA) (0.1 µg ml^{-1}) for 20 min.

Spermatogenesis is of the hologenous type where both daughter nuclei survive and equal cytokinesis occurs at both meiosis I and meiosis II (ref. 24). Spermatogenesis begins with a series of differentiating or special mitosis, also known as the 'depauperating mitosis'[4,6]. The vegetative cell that differentiated to initiate spermatogenesis (also called the spermatogonangium) was divided into two equal halves following the first depauperating mitosis. This takes place in the median valve, wherein a rudimentary theca is placed (Figure 1 *b*), separating the spermatogonangium into two half-spermatogonangia[4]. The cell undergoes three additional, successive, depauperating mitoses producing a total of 16 spermatogonia per cell, i.e. 8 spermatogonia per half-spermatogonangium

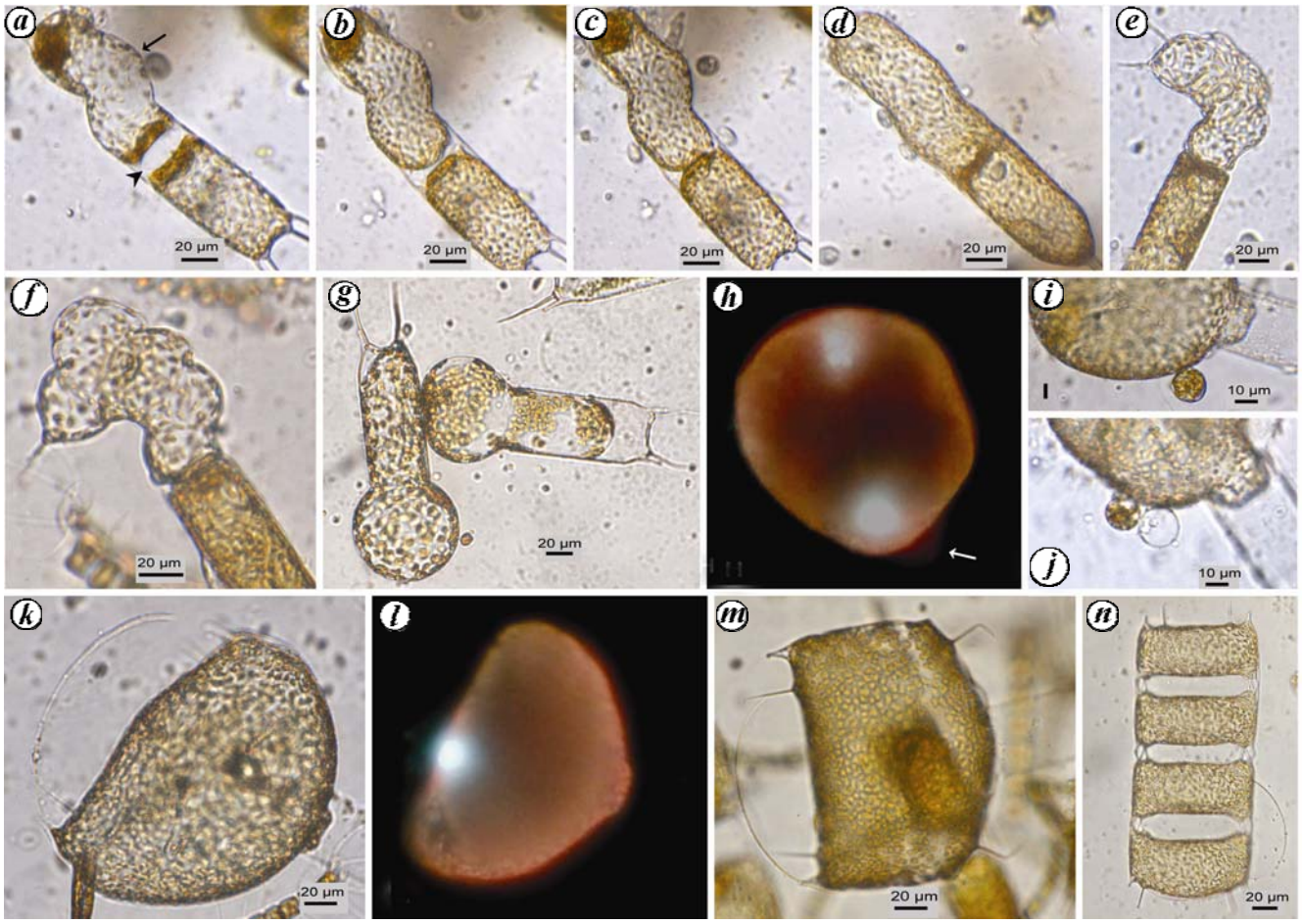

Figure 2. *Odontella regia*. ***a***–***n***, Oogenesis, fertilization and auxosporulation. ***a***, The cell acquires a hump (arrow) following plasmolysis (arrowhead), but without cell division. ***b***–***d***, Sequence of plasmogamy. ***e***, ***f***, Development seen in the hump, the complete process could not be captured (see text). Therefore, oocytes formed elsewhere in the culture well have been photographed (***g***). ***h***, DAPI-stained, unfertilized egg with two nuclei and a sperm (fluorescing red) attached (arrow). ***i***, ***j***, Fertilization. ***i***, Sperm attaches to oocyte and transfers nuclear content. ***j***, Transfer complete, a second sperm that has attached itself to the oocyte later degenerates. ***k***, Enlargement of auxospore. ***l***, DAPI-stained auxospore showing diploid nucleus. ***m***, First filial generation (first cell). ***n***, The first cell has undergone two mitotic divisions, resuming the vegetative mode of life.

(Figure 1 *c*). The spermatogonium undergoes two meiotic divisions to produce four flagellated sperms each (Figure 1 *f*). Therefore, 32 sperm cells per half-spermatogonangium, i.e. 64 sperm cells per cell were produced. It was observed that the two half-spermatogonangia did not develop together. The spermatogonium in one half-spermatogonangium developed first into the flagellated sperm, which immediately began to move within the cell. This activity caused dehiscence and release of sperms. The total time taken for one half-spermatogonangium to release sperm cells was 2 h : 31 min : 46 s.

Oogonium developed directly from vegetative cells; the oogonium itself became the oocyte. *O. regia* undergoes type-1 oogenesis[24]. In this type, two secondary oocytes, each containing a functional haploid nucleus, are produced by nuclear division and equal cytokinesis at meiosis I. Meiosis II follows in each secondary oocyte with no cytokinesis and one of the two daughter haploid nuclei produced by this nuclear division becomes pycnotic in each secondary oocyte. Finally the secondary oocytes, each containing a functional nucleus and a pycnotic nucleus[24] (Figure 2 *h*), become eggs at the open ends of the thecae (Figure 2 *g*). Eggs were buoyant and therefore floated in the culture well. Due to the rarity of oogenesis in our culture, the complete sequence of oogenesis could not be recorded. The cells that were being followed to record the sequence of oogenesis stopped developing/aborted at crucial points. Elsewhere in the culture well, well-formed oocytes (Figure 2 *g*) were recorded; an assumption, that the strong light of the microscope inhibited the formation of the oocyte, may be made. The images represented (Figure 2 *a*–*f*) may perhaps be the development of the oocyte. On DAPI staining, most of the already formed oogonial cells showed the presence of two nuclei (Figure 2 *h*). These seemed to represent the sister nuclei from meiosis II. We had initially assumed the second nuclei to be the sperm nucleus, but further observations of other oogonial cells confirmed the presence of the second (pycnotic) nucleus derived after meiosis II, since a faint nuclear thread between the two daughter nuclei was observed (could not be photographed clearly). This indicated that meiosis was complete before

fertilization, unlike the observation of oogenesis in another centric diatom *Thalassiosira punctigera*, where meiosis was incomplete at fertilization and meiosis II occurred after fertilization[7].

The released sperms swimming actively in the media were found attached to the respective eggs. Fertilization occurred with the contents of the sperm cell being completely transferred into the egg, leaving behind only an empty frustule (Figure 2 *i*, *j*) (in some cases two or three sperm cells would attach to an oocyte, although only one sperm successfully fertilized an egg). This process took 24 h. An organic wall was formed within 6 h of fertilization forming the auxospore. DAPI staining of auxospores (Figure 2 *l*) showed a single diploid nucleus. Gradually the auxospore changed shape from spherical to a slightly compressed ellipsoid (Figure 2 *m*) and underwent the first mitotic division and successively the second mitotic division (Figure 2 *n*), entering the cycle of asexual reproduction.

The trans-apical valve lengths of 25 randomly selected cells (male determined, female determined and the F_1 generation (progeny), each) were measured. Cells in the process of spermatogenesis and cells attached to the auxospores were measured for male and female cell sizes respectively. The trans-apical valve length in male cells was 25–32 μm (SD ± 2.07), and that of the female cells varied from 30 to 50 μm (SD ± 6.37). The F_1 generation had a size range between 50 and 145 μm, and a large standard deviation (± 20.43). Jewson[10] observed that the size of the auxospore is proportionate to the size of the oocyte, and also that the size of the auxospore may be dependent on the internal reserves of the mother cell.

This strain of *O. regia* recorded from Dona Paula Bay had an apical length of 137 μm and trans-apical length of 37 μm. The first cell was about 40 μm in length. This species gained breadth after sexual reproduction and the trans-apical length was measured to be 115 μm.

The course of gametogenesis not only varies at the intergeneric level, but also at the interspecific level, it is interesting to note that three closely related diatom taxa of similar habit and morphology that undergo type-I oogenesis and hologenous-type spermatogenesis[24], viz. *O. sinensis*, *O. regia* and *O. mobiliensis*, have three different sperm cell counts. Drebes[14] reported the number of sperms for *O. sinensis* and *O. mobiliensis* as 128 and 32 respectively. However, the sperm count of *O. regia* was not reported. With the report of 64 sperm cells in *O. regia*, this finding fills the void.

1. MacDonald, J. D., On the structure of the diatomaceous frustule and its genetic cycle. *Ann. Mag. Nat. Hist.*, 1869, **3**, 1–8.
2. Pfitzer, E., Uber den Bau und Zellteilung der Diatomeen. In *Bot. Zeitung* (ed. Hanstein), 1869, **27**, 774–776.
3. Lewis Jr, W. M., The diatom sex clock and its evolutionary significance. *Am. Nat.*, 1984, **123**, 73–80.
4. Drebes, G., Sexuality. In *The Biology of Diatoms* (ed. Werner, D.), Blackwell Scientific Publications, Oxford, 1977, pp. 250–283.
5. Edlund, M. B. and Stoermer, E. F., Ecological, evolutionary, and systematic significance of diatom life histories. *J. Phycol.*, 1997, **33**, 897–918.
6. Chepurnov, V. A., Mann, D. G., Sabbe, K. and Vyverman, W., Experimental studies on sexual reproduction in diatoms. *Int. Rev. Cytol.*, 2004, **237**, 91–154.
7. Chepurnov, V. A., Mann, D. G., von Dassow, P., Armbrust, V. E., Sabbe, K., Dasseville, R. and Vyverman, W., Oogamous reproduction, with two-step auxosporulation, in the centric diatom *Thalassiosira punctigera* (Bacillariophyta). *J. Phycol.*, 2006, **42**, 845–858.
8. Mann, D. G., Why didn't Lund see sex in *Asterionella*? A discussion of the diatom life cycle in nature. In *Algae and the Aquatic Environment* (ed. Round, F. E.), Biopress Ltd, Bristol, UK, 1988, pp. 384–412.
9. Jewson, D. H., Life cycle of a *Stephanodiscus* sp. (Bacillariophyta). *J. Phycol.*, 1992, **28**, 856–866.
10. Jewson, D. H., Size reduction, reproductive strategy and the life cycle of a centric diatom. *Philos. Trans. R. Soc. London, Ser. B.*, 1992, **335**, 191–213.
11. Thwaites, G. H. K., On conjugation in the Diatomaceae. *Ann. Mag. Nat. Hist.*, 1847, **20**, 343–344.
12. Schmidt, P., Ist die scharfe trennung zwischen zentrichen und pennaten diatomeen halbar? *Int. Rev. Ges. Hydrobiol.*, 1927, **18**, 274–288.
13. Von Stosch, H. A., Oogamy in a centric diatom. *Nature*, 1950, **165**, 531–532.
14. Drebes, G., *Marines Phytoplankton. Eine Auswahl der Helgolander Planktonalgaen* (*Diatomeen, Peridineen*), Thieme, Stuttgart, 1974, p. 186.
15. Mann, D. G., Patterns of sexual reproduction in diatoms. *Hydrobiologia*, 1993, **269/270**, 11–20.
16. Koester, J. A., Brawley, S. H., Karp-Boss, L. and Mann, D. G., Sexual reproduction in the marine centric diatom *Ditylum brightwellii* (Bacillariophyta). *Eur. J. Phycol.*, 2007, **42**, 351–366.
17. Subrahmanyan, R., On the occurrence of microspores in some centric diatoms of the Madras coast. *J. Indian Bot. Soc.*, 1946, **25**, 61–66.
18. Subrahmanyan, R., On somatic division, reduction division, auxospore-formation and sex differentiation in *Navicula halophila* (Grun.) CL. *J. Indian Bot. Soc. (M.O.P. Iyengar Commemorative volume)*, 1946, 239–266.
19. Rao, V. N. R. and Desikachary, T. V., MacDonald–Pfitzer hypothesis and cell size in diatoms. *Nov. Hedw. Beih.* 1970, **31**, 485–493.
20. Ragothaman, G. and Rao, V. N. R., Studies on the diatom *Amphora coffeaeformis* Agardh: salinity changes on growth and auxospore formation. *Indian J. Mar. Sci.*, 1978, **7**(1), 62–65.
21. Mayer, C. and Schmid, A.-M. M., Morphology, cell-cycle and growth-rates of *Odontella regia*. *Diatom Res.*, 1995, **10**(2), 299–320.
22. Tomas, C. R., *Identifying Marine Phytoplankton*, Academic Press, San Diego, California, 1997, p. 858.
23. Guillard, R. R. L. and Ryther, J. H., Studies of marine planktonic diatoms I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.*, 1962, **8**, 229–239.
24. Mizuno, M., Evolution of meiotic patterns of oogenesis and spermatogenesis in centric diatoms. *J. Phycol. Res.*, 2006, **54**, 57–64.

ACKNOWLEDGEMENTS. We thank Dr S. R. Shetye, Director, National Institute of Oceanography, Goa for support. S.H. is grateful to Dr S. Raghukumar for encouragement. We thank Dr M. P. Tapaswi, K. Venkat and Amey Kinalekar for help during the preparation of this manuscript. S.H. and D.D.N. thank their colleagues from MCMRD for their cooperation, suggestions and help. This work was funded by the Ballast Water Management Programme, India. This is an NIO contribution no. 5003.

Received 21 March 2011; accepted 6 June 2011