A NEW GENUS, *PIERRECOMPERIA* GEN. NOV., A NEW SPECIES AND TWO NEW COMBINATIONS IN THE MARINE DIATOM FAMILY CYMATOSIRACEAE

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ABSTRACT. – A new, monospecific diatom genus, *Pierrecomperia* gen. nov. (typus generis *P. catenuloides*), is described. In addition, a new species is described in the genus *Cymatosira* Grunow, viz. *C. minutissima*. We also propose two new combinations in the genus *Plagiogrammopsis* Hasle, von Stosch & Syvertsen, namely *P. minima* comb. nov. and *P. sigmoidea* comb. nov. (both formerly placed in *Plagiogramma* Greville). *Plagiogramma parallelum* Salah, *P. minimum* Salah and *P. sigmoideum* Salah are lectotypified. *P. parallelum* is synonymized with *Brockmanniella brockmannii* (Hustedt) Hasle, von Stosch & Syvertsen. Morphological and molecular (18S rDNA and *rbc*L) evidence firmly places *Pierrecomperia* in the centric diatom family Cymatosiraceae, which is uniquely characterized by the presence of ocelluli. Like *Extubocellulus* and *Pseudoleyanella*, *Pierrecomperia* is isovalvar and is therefore placed in the subfamily Extubocelluloideae. Like most members of the diatom family Cymatosiraceae, the above-mentioned taxa are confined to coastal shallow water habitats where they have adopted a benthic or tychoplanktonic life-form. Their biogeography and autecology is discussed.

INTRODUCTION

The centric diatom family Cymatosiraceae was established by Hasle, von Stosch and Syvertsen in 1983 to accommodate the known genera *Cymatosira* Grunow and *Campylosira* Grunow ex Van Heurck, and seven newly described genera, viz. *Plagiogrammopsis* and *Brockmanniella* (both formerly in *Plagiogramma* Greville), *Minutocellulus* and *Extubocellulus* (both formerly in *Bellerochea* Van Heurck), *Leyanella*, *Arcocellulus*, and *Papiliocellulus* (Hasle *et al.* 1983). A tenth genus, *Pseudoleyanella*, was described by Takano (1985). On the basis of the structure of the auxospore envelope and the discovery of flagellate male gametes in some taxa, it was recognized that the Cymatosiraceae belong to the centric diatom lineage. They are characterized by a number of morphological cell wall features, the most important being the structure of the valve apex (with ocelluli, elevated pore fields surrounded by a distinct rim, Hasle *et al.* 1983) and the occurrence of tubular processes, pili and quasifract bands in some genera (cf. Hasle *et al.* 1983). All taxa have a single, plate-like plastid (Hasle *et al.* 1983). Two subfamilies are recognized, the Cymatosiroideae Hasle, von Stosch & Syvertsen, which is heterovalvate and the Extubocelluloideae Hasle, von Stosch & Syvertsen, which is isovalvate. *Lennoxia*, a monotypic genus described by

Thomsen *et al.* (1993), might also belong to the Cymatosiraceae (Hasle & Syvertsen 1996): it is heterovalvar with one subcentral tubular process per cell but the presence of ocelluli could not be verified. Three extinct genera, *Rossiella* Desikachary & Maheshwari, *Bogorovia* Jousé and *Koizumia* Yanagisawa (based on *Cymatosira adaroi* Azpeitia y Moros), also belong to the family Cymatosiraceae according to Yanagisawa (1996), although they do not possess a typical ocellulus. Morphological and molecular-genetic analyses place representatives of the Cymatosiraceae as a monophyletic clade in the bi- and multipolar centrics (see e.g. Medlin & Kaczmarska 2004).

Except for *Cymatosira*, which contains about 17, mainly fossil species (VanLandingham 1967-1979), many of which have not been revised yet, most genera comprise only a few species or are monospecific (*Brockmanniella*, *Leyanella* and *Pseudoleyanella*). In the present study, five new taxa and combinations are proposed in the Cymatosiraceae, viz. the genus *Pierrecomperia* gen. nov. and the species *P. catenuloides* sp. nov., *Cymatosira minutissima* sp. nov., and the new combinations *Plagiogrammopsis minima* comb. nov. and *P. sigmoidea* comb. nov. The latter is proposed as the correct name for *P. mediaequatus* Gardner & Crawford. *Plagiogramma parallelum* and the basionyms of *Plagiogrammopsis minima* and *P. sigmoidea* are lectotypified.

MATERIALS AND METHODS

Surface sediment samples were collected from intertidal mud- and sand flats along the entire salinity gradient (eu- to oligohaline) in the Westerschelde estuary (The Netherlands, between 1992-2009) and meso- to polyhaline transition zone in the Tagus estuary (Portugal, 2002-2004). The Westerschelde and Tagus sediment samples were fixed with formalin (4 %) and gluteraldehyde (2.5 %) respectively. Cleaned frustules for microscopic analysis [both light microscopy (LM) and scanning electron microscopy (SEM)], were obtained by treating the samples with nitric acid (70 %) and sulphuric acid (99 %) followed by gentle heating, or with hydrogen peroxide (35 %). Permanent preparations were made with Naphrax mounting medium and examined using a Leitz Diaplan microscope with Differential Interference Contrast (DIC) optics or a Zeiss Axioskop 50 optical microscope, equipped with Bright field, Phase Contrast and DIC optical microscopy (LM). Cleaned material was also air-dried onto stubs and sputter-coated with gold for SEM; SEM observations were performed with a Jeol JSM-840 (at 15 kV) and a Hitachi S 4500. Live and fixed material was examined for information on life-form, plastid structure and microhabitat. Frustule dimensions (length, width and number of areolae in 10 μm) were determined for each species; averages and standard deviations are given (n = 20 unless stated otherwise). Terminology used in the description of the structures of the siliceous cell wall is based on Anonymous (1975), Ross et al. (1979), Hendey (1964: valve outlines and structural types), Cox & Ross (1980) and Round et al. (1990: cingulum and raphe structures).

We also examined Salah's material from Blakeney Point (Norfolk, UK, Salah 1953, 1955) on BM slides 36399-36402 and 36405-36407, and Hustedt's material from the German Wadden Sea (BRM slide N12/36, Leybucht, Leysand: Hustedt 1939). Lectotype slides are proposed for *Plagiogramma minimum*, *P. parallelum* and *P. sigmoideum*, three species described by Salah (1955) for which no holotype slides were indicated.

An intertidal sediment sample containing living cells of *Pierrecomperia catenuloides* was collected on 2 March 2009 at Rammekenshoek (51°26'54" N, 3° 38'51"S) in the polyhaline part of the Westerschelde estuary (The Netherlands). A small aliquot of sand grains was transferred into sterile natural seawater collected from the North Sea enriched with F/2 nutrients (Guillard 1975). Two clonal cultures of *Pierrecomperia catenuloides* and one culture of *Leyanella arenaria* were established by isolation of single cells by micropipette into multiwell plates (Greiner Bio-One, Frickenhausen, Germany) containing F/2 culture medium. The cultures were kept in an incubator at 19°C with a 12:12 light:dark period and 50 μmol photons m^{-2} s^{-1} from cool-white fluorescent tubes. The cultures were transferred every 10 days to fresh medium.

Cells for DNA extraction were harvested from exponentially growing cultures and pelleted by centrifugation. For the amplification and sequencing of the *rbc*L gene, DNA was extracted using the bead-beating method with phenol extraction and ethanol precipitation as described by Zwart et al. (1998). The *rbc*L gene was amplified in a PCR with a degenerate primer pair as described in Wawrik et al. (2002). PCR mixtures (50 μl) contained 1μL of template DNA, primers at 0.5 μm each, dNTPs at 0.2 mM, 1.5 mM MgCl$_2$, 2.5 U of Taq DNA polymerase and PCR buffer (Qiagen). PCR reaction cycles were as described in Wawrik et al. (2002). For the 18S rRNA gene, forward primers were DDSU4 (5' AACCTGGTTGATTCTGCCAG-TAG-3'), DSSU550 ('5-AAGTCTGGTGCCAGCAGCC-3'), and DSSU1119 (5'-GGCTGAAACTTAAAGAAATTG-3'). Reverse primers were DDSU376 (5'-TCTCAGGCTCCCTCTC-CG-3'), DSSU1180 (5'-TCCACCAACTAAGAACGGCC-3'), DSSU1613 (5'-GTACAAAGGGCAGGGACGTA-3') and DSSU1860 (5'-CTGCAGGTTCACCTACGGAAACC-3'). For the PCR, the reaction mixture contained 1-5 μL of template DNA, dNTPs at 0.2 mM, primers at 1 μm each, and 2.5 U Taq polymerase and PCR buffer was adjusted to a total volume of 50 μL with sterile water. PCR reaction cycles were as described in Vanelslander et al. (2009).

Sequences (of both the *rbc*L and the 18S rRNA genes) were obtained with the ABI 3100 prism® BigDye® Terminator Cycle Sequencing Ready Reaction Kit. Sequences were edited separately using BioNumerics version 3.5 (Applied Maths, Kortrijk, Belgium). *Rbc*L sequences from *Pierrecomperia catenuloides* and 31 other diatom species and 2 *rbc*L sequences of *Bolidomonas* available in Genbank were automatically aligned using BioEdit. For the 18S rRNA gene sequences, we used the alignment created by Theriot et al. (2009), which was aligned according to secondary structure, and manually added the 18S sequences of *Pierrecomperia catenuloides*. Accession numbers for the new sequences are given in Figs. 48 and 49. Phylogenetic analyses on the *rbc*L and 18S rRNA gene were performed using Bayesian inference (BI) in MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003). The model used was GTR+I+G. No initial values were assigned to the model parameters. Two runs of four Markov Chains (one cold and three heated) were run for three million generations and sampled every 100 generations. This yielded a posterior probability distribution of 30,001 trees. After exclusion of 5000 'burn-in' trees, posterior probabilities were calculated by constructing a 50 % majority-rule consensus tree.

Autecological information on the species described below is based on analyses of their occurrence in (1) a large data set comprising quantitive cell counts of 185 Westerschelde samples taken during the period October 1991-October 1992 (Sabbe 1997) and (2) a data set comprising 6 stations sampled bimonthly between 2002 and 2004 (see Jesus et al. 2009 for more details). Sediment grain size parameters, interstitial water content and salinity, and organic matter content were determined on all sampling occasions (see Sabbe 1997 and Jesus et al. 2009 for methodology).

RESULTS

Family Cymatosiraceae Hasle, von Stosch & Syvertsen

Subfamily Cymatosiroideae Hasle, von Stosch & Syvertsen

Brockmanniella brockmannii (Hustedt 1939) Hasle, von Stosch & Syvertsen 1983 (Figs. 12, 13, 39)

Synonyms: *Plagiogramma brockmannii* Hustedt 1939, *Plagiogramma parallelum* Salah 1955, (?) *Cymatosira capensis* Giffen 1975

A detailed description of this species is given by Hasle *et al.* (1983) and Gardner & Crawford (1994). The genus *Brockmanniella* is characterized by the presence of a fascia, marginal, granule-like spines and a subcentral rimoportula; it has no pili or pseudosepta. *Brockmanniella brockmannii* appears to be conspecific with *Plagiogramma parallelum*, which was described by Salah (1955) from Blakeney Point (Figs. 12, 13). The latter species has more or less parallel valve margins and is on average slightly broader (5-6 µm) than *B. brockmannii*. However, as shape and dimensions fall within the range of morphological variation of *B. brockmannii* (Gardner & Crawford 1994) and no other differences exist, we consider both species to be conspecific. As *Plagiogramma parallelum* was never typified, we propose the following lectotype slide: Blakeney 5B, 1949, BM 36399, design. Sabbe K.

According to Hasle *et al.* (1983) *B. brockmannii* is also conspecific with *Cymatosira capensis* Giffen described from Saldanha bay (South Africa, Giffen 1975). The latter species, however, does not appear to have the large fascia which is typical of *B. brockmannii*. It remains to be assessed whether this area was not observed by Giffen or whether it is truly absent, in which case *C. capensis* might still constitute a separate species (see also below).
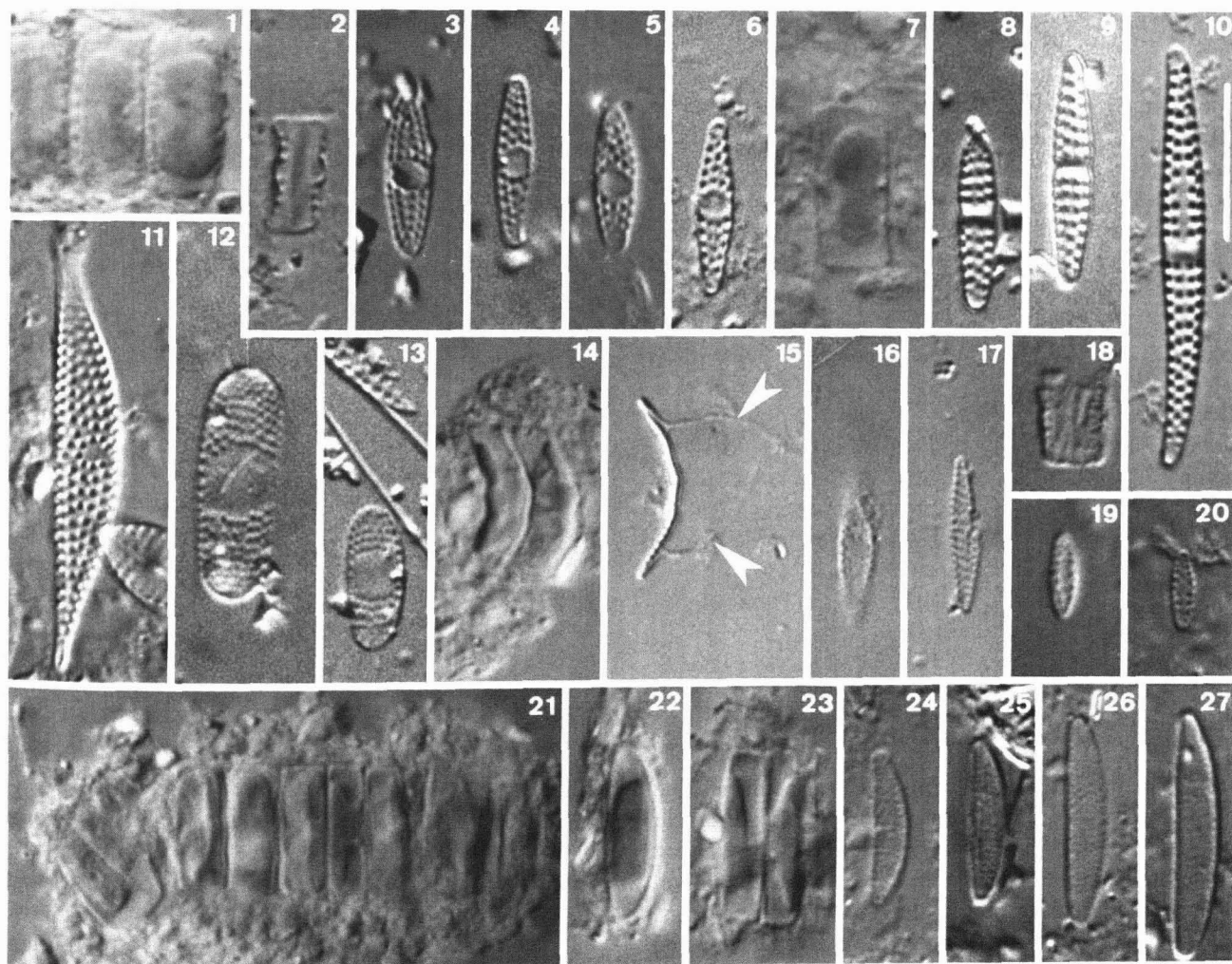
Figs 1-27. – Different species belonging to the diatom family Cymatosiraceae. Light microscopy, differential interference contrast. All photographs are at the same magnification [scale bar in Fig. 10 represents 10 µm] and concern valve views unless stated otherwise. Figs 1-2, 6-7, 10-11, 14-24, 26-27. Westerschelde estuary (The Netherlands). Figs 3-5, 8-9, 12-13: Blakeney Point (England), BM slides 36399, 36401, 36402. Fig. 25: Leybucht, Wadden Sea (Germany), BRM slide N12/36. Fig. 1. *Cymatosira belgica* Grunow. Living material; small colony in girdle view. Figs 2-6. *Plagiogrammopsis sigmoidea* (Salah) Sabbe & Muylaert comb. nov. Fig. 2. Girdle view. Figs. 7-10. *Plagiogrammopsis minima* (Salah) Sabbe & Witkowski comb. nov. Fig. 7. Living cell in girdle view. Fig. 11. *Campylosira cymbelliformis* (A. Schmidt) Grunow. Figs 12-13. *Brockmanniella brockmannii* (Hustedt) Hasle, von Stosch & Syvertsen. Figs 14-16. *Leyanella arenaria* Hasle, von Stosch & Syvertsen. Fig. 14. Two living cells in girdle view. Fig. 15. Single cell in girdle view. Arrowheads indicate the pili. Figs 17-20. *Cymatosira minutissima* Sabbe & Muylaert sp. nov. Fig. 18. Girdle view. Figs 21-27. *Pierrecomperia catenuloides* Sabbe, Vyverman & Ribeiro gen. et sp. nov.

Biogeography and ecology: *Brockmanniella brock-mannii* is a common species in sediments and plankton in North Europe (Hasle & Syvertsen 1996) but was also found in samples from Florida (USA) and is probably much more widespread (Round *et al.* 1990). It is common in silty sediments in the polyhaline reaches of the Westerschelde estuary, which is in accordance with its occurrence in the plankton of these reaches (Muylaert & Sabbe 1999).

Cymatosira minutissima Sabbe & Muylaert sp. nov.
(Figs. 17-20, 28-31)

Descriptio : cellulae minutae, facie connectivali rectangulares. Valvae ellipticae-lanceolatae, polis rotundatis vel cuneatis, 2.5-10 μm (5.8 ± 2.0) longae, 1.5-2.5 (1.9 ± 0.3) μm latae, sine fascia. Frons valvae valde curvata in sectione transapicali ; limbus profundus. Areolae 18.5-24 in 10 μm (21.3 ± 1.7), cribris externis occlusae. Ocelluli porellis centralibus 2-4 leviter diagonaliter oppositae. Spinae simplices probabiliter praesentiae junctura frontis cum limbo. Cingulum profundus ex copulis numerosis constans. Habitat in mari, probabiliter ad floram interstitialem vel affixam sabuletorum.

Description: cells very small, rectangular in girdle view. Valves elliptical to lanceolate, with rounded to cuneate apices, 2.5-10 (5.8 ± 2.0) μm long, 1.5-2.5 (1.9 ± 0.3) μm wide. Fascia absent. Valves strongly curved in transapical section, mantle deep. Areolae 18.5-24 in 10 μm (21.3 ± 1.7), occluded by external cribra. Ocelluli two, opening slightly laterally, with 2-4 central porelli. Marginal spines present but rare. Cingulum wide, composed of numerous copulae. Probably belongs to the interstitial or attached flora of marine sand flats.

Holotypus: BM slide 101463, The Natural History Museum, Department of Botany, London (BM)

Isotype: slide KS1001, The Herbarium, Ghent University (GENT).

Type locality: sandy beach (median grain size 305 μm) at Vlissingen (Ritthem) near the mouth of the Westerschelde estuary (The Netherlands); salinity range 27.5-33 ‰ (30.3 ± 2.5).

Comment: no distinct hyaline areas are present. The areolae are arranged in longitudinal rows along the valve margin (especially in the larger specimens, Fig. 17) and are more irregularly placed in the centre of the valve. The ocelluli are only slightly diagonally opposed (Figs. 28, 29). No spines are present on the external cribra and around the ocelluli. The valve face is curved; the centre of the valve is distinctly bulged (Fig. 28). The mantle is rather deep. In one specimen two small marginal spines can be seen (Fig. 28).

Cymatosira minutissima was assigned to the genus *Cymatosira* mainly on the basis of negative features: it lacks a fascia or pseudoseptum and has no pili or marginal ridges (thus ruling out the genera *Plagiogrammop-*

sis, Brockmanniella, Minutocellulus, Papiliocellulus, Arcocellulus, Leyanella and *Pseudoleyanella*). It has a different girdle structure and pervalvar/apical axis ratio than representatives of the genus *Extubocellulus* and its valves are also not sub-lunate as in the genus *Campylosira* (Hasle *et al.* 1983). However, the affinity of this species with the genus *Cymatosira* is also not readily visible: it lacks a number of characteristic generic features, such as a subcentral rimoportula (on process valves) or a well-developed ring of interlocking marginal spines. As none of the valves observed in SEM shows these features, it seems unlikely that specialized end valves (with larger ocelluli, simple spines and a prominent rimoportula) or process valves were simply overlooked during this study. A more plausible explanation for the absence of these structures lies in the overall size of *Cymatosira minutissima* (see discussion).

In LM, *Cymatosira minutissima* can sometimes be confused with *Leyanella arenaria*. However, the latter species has a strongly curved valve which can readily be seen in LM, both in valve and girdle view (Figs. 14-16). In addition, *Leyanella arenaria* has distinct pili (Fig. 15), marginal ridges and a subcentral tubular process (Hasle *et al.* 1983).

Cymatosira minutissima is also quite reminiscent of *Cymatosira capensis* Giffen (Giffen 1975) but has a less distinctly undulated valve face (cf. Giffen 1975, Figs. 36, 37) and a higher areolar density. Examination of Giffen's type material is necessary to assess the relationship between both taxa but to date we have been unable to get access to Giffen's type material.

It is not unlikely that the specimens illustrated as *Plagiogrammopsis crawfordii* Witkowski, Lange-Bertalot & Metzeltin in Witkowski *et al.* (2000) on Pl. 12, Figs. 15-18 belong to *Cymatosira minutissima*. Unfortunately, the protologue of *P. crawfordii* and the specimens illustrated do not agree (see below), and as no specimens from the holotype locality were illustrated, its true identity remains uncertain.

Biogeography and ecology: In the Westerschelde estuary *Cymatosira minutissima* was mainly found in fine and medium sandy sediments [in contrast with *C. belgica* which predominantly occurs in silty sediments and in the plankton (Muylaert and Sabbe 1999)]. It is not sure whether it belongs to the interstitial flora or whether it can also be attached to sand grains. *C. minutissima* is most abundant during the summer months. The distribution of *C. minutissima* is as yet unknown but given its minute size it has probably often been overlooked.

Plagiogrammopsis minima (Salah 1955) Sabbe & Witkowski comb. nov. (Figs. 7-10, 32-35, 43-44)

Basionym: *Plagiogramma minimum* Salah 1955, Hydrobiologia 7, p. 91, Pl. I, Fig. 15.
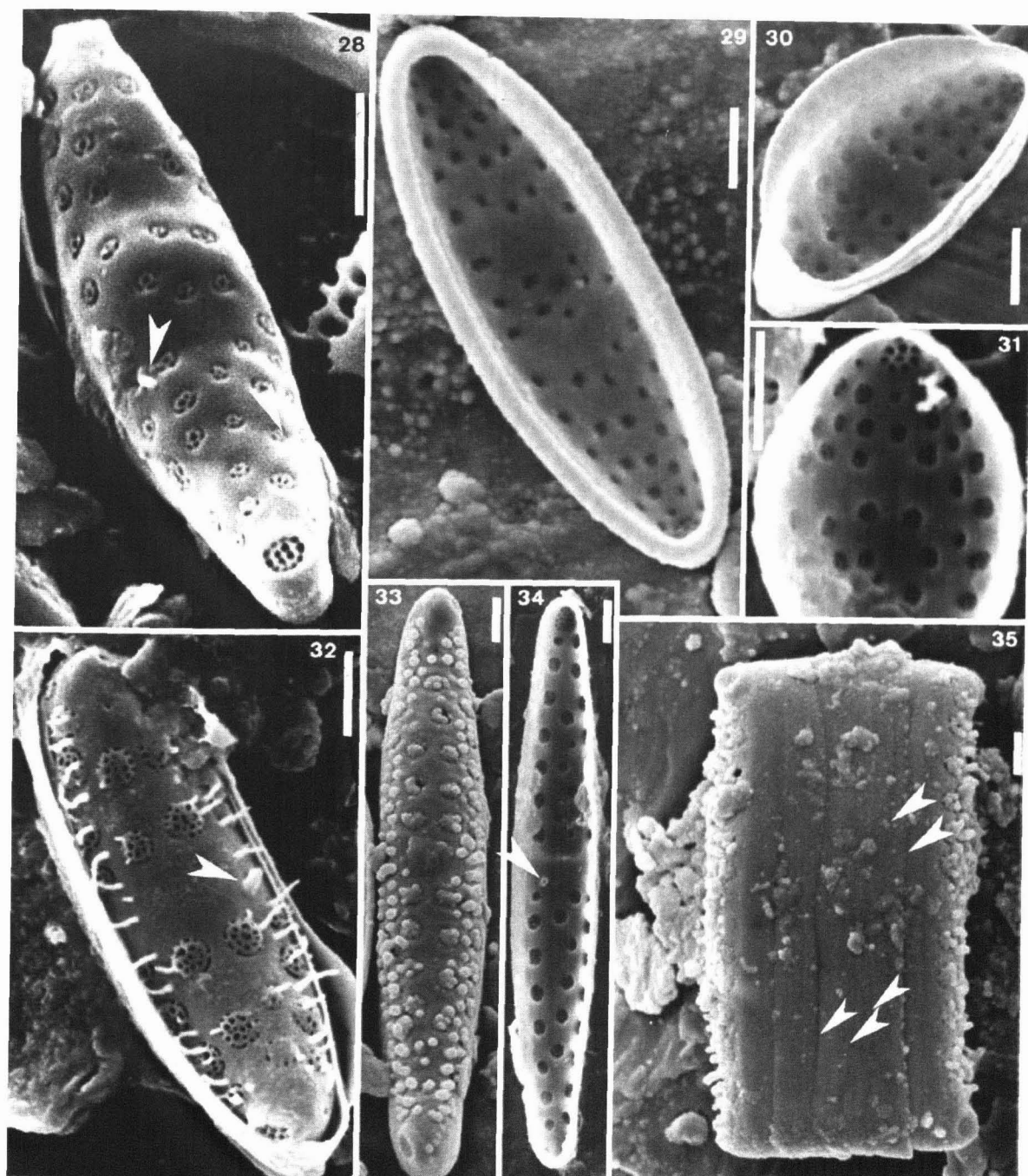
Figs 28-35. – *Cymatosira minutissima* and *Plagiogrammopsis minima*, SEM, samples from the Westerschelde estuary (The Netherlands). Scale bars represent 1 μm. Figs 28-31. *Cymatosira minutissima*. Fig. 28. External valve view. Note the presence of two small spines (arrows). Figs 29-31. Internal valve views. Figs 32-35. *Plagiogrammopsis minima*. Figs 32-33. External valve views. In Fig. 32 the external tubular opening of the process is visible (arrow). Fig. 34. Internal valve view. Note the central, offset process (arrow). Fig. 35. External girdle view. Five non-porous copulae can be seen (arrows).

Lectotype: Blakeney 5B, 1949, BM 36399, design. Sabbe K.

Synonym: *Plagiogrammopsis crawfordii* pro parte (Witkowski *et al.* 2000, Pl. 10, Figs. 37-39).

Comment: the cells of *Plagiogrammopsis minima* are solitary or form short colonies of 2-3 cells; they are attached to each other and to sand grains at the poles. There is one single, in girdle view slightly butterfly-shaped plastid (Fig. 7), which appears to be appressed

to one side of the girdle, as in *Plagiogrammopsis van-heurckii* (Hasle *et al.* 1983, Fig. 104). The frustules are rectangular in girdle view, about 4-4.5 μm wide; they are centrally slightly constricted and have raised but blunt apices (Figs. 7, 35). The valves are lanceolate with rounded, sometimes slightly produced apices, 6.4-16 μm long and 1.9-3.2 μm wide (Westerschelde, n = 29). One single, very large (on average 27 μm, initial valve?) valve is slightly curved at the apices (Fig. 10). No sterna are vis-
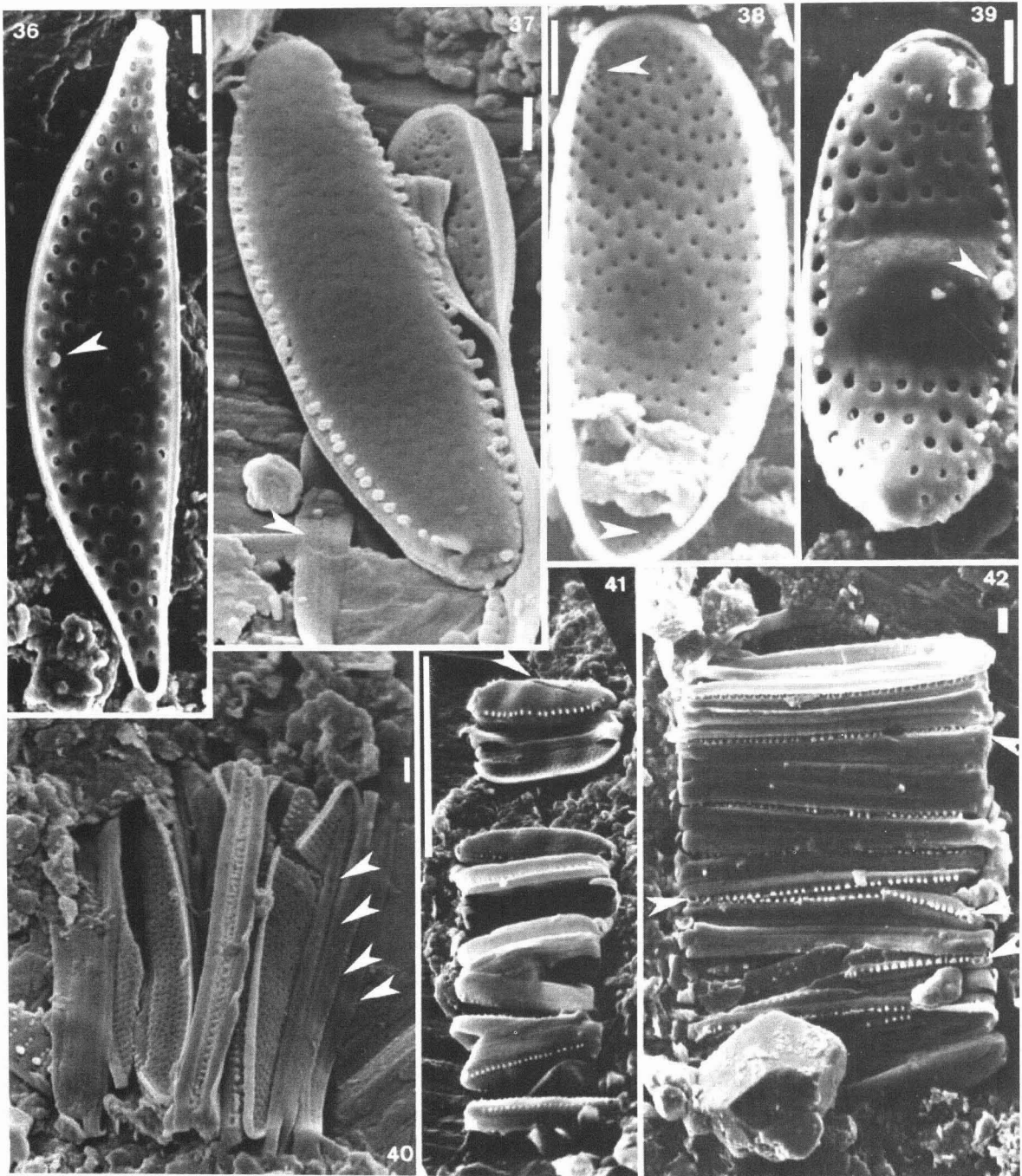
Figs 36-42. – *Campylosira cymbelliformis*, *Pierrecomperia catenuloides* and *Brockmaniella brockmannii*, SEM, samples from the Westerschelde estuary (The Netherlands). Scale bars represent 1 μm, except in Fig. 41 where it represents 10 μm. Fig. 36. *Campylosira cymbelliformis*. Internal valve view showing the central submarginal process (arrow). Fig. 37-38. *Pierrecomperia catenuloides*. Fig. 37. External valve view. Note the porous copulae (arrow). Fig. 38. Internal valve view with arrowheads indicating the diagonally opposed ocelluli. Fig. 39. *Brockmaniella brockmannii*. External valve view. The external tubular opening of the process is arrowed. Figs 40-42. *Pierrecomperia catenuloides*. External girdle views showing spine-mediated colony formation. Note the presence of 4 copulae in Fig. 40 (arrows), the bulged valve centre in Fig. 41 (arrow) and the distinctly rimmed ocelluli in Fig. 42 (arrows).

ible. The valve face is curved, merging almost impercep-tibly with a rather deep mantle. The centre of the valve appears slightly but distinctly constricted in LM (Figs. 7-10). Internally, a pseudoseptum runs from margin to margin in the central fascia (Figs. 34, 43, 44). On one side of this pseudoseptum, a slightly offset process which opens externally via a short tube is visible (Figs. 32, 34,

44), but is not always present (Fig. 43). The areolae (11-16, mostly 12-14 in 10 μm, Westerschelde, n = 29) along the valve margin are arranged in longitudinal rows; on the rest of the valve, their pattern is rather irregular. The areolae are more or less round and occluded by external, cribrate vela (with about 8-10 perforations each, Figs. 32, 34, or less, Figs. 43, 44). Externally, spinules can be pres-

ent on the cribra (Fig. 32). No areolae are present at the apices or in the centre of the valve. At each pole, a raised ocellulus is present; they open laterally in opposite directions and are distinctly diagonally arranged in a clockwise direction (Figs. 32-35, 43, 44). A marginal ring of rather long, thin spines is present (Fig. 32). The cingulum consists of 5 open copulae in at least one specimen; no perforations are visible (Fig. 35).

The above-described diatom does not belong to the genus *Plagiogramma* Greville which has large ocelli and different plastid structure and stria patterns. *Plagiogramma* is not heterovalvous and has no processes (Hasle *et al.* 1983, Round *et al.* 1990). We propose a transfer to the genus *Plagiogrammopsis*. Although no pili were observed, this transfer is justified as all other characteristics of this species fit the generic description of *Plagiogrammopsis* (viz. plastid structure, presence of a pseudoseptum, long spines, distinctly laterally opening ocelluli and an external cribrum with spinules). The closely related genus *Brockmanniella* does not possess a pseudoseptum or external cribra with spinules, while *Cymatosira* has no fascia or pseudoseptum and has different spines (Hasle *et al.* 1983) (Table 2). *P. minima* differs from *P. sigmoidea* (cf. below) in the presence of a pseudoseptum (Gardner & Crawford 1994).

As mentioned above, the exact identity of *Plagiogrammopsis crawfordii* is unclear. The specimens illustrated in Witkowski *et al.* (2000) on Pl. 10, Figs. 37-39 fully correspond to *P. minima*. These specimens have no more than 14 areolae in 10 μm and therefore do not correspond to the protologue of *P. crawfordii*, which mentions 30 areolae in 10 μm. The specimens illustrated on Pl. 12, Figs. 15-18 belong to a different taxon (see above).

Biogeography and ecology: *Plagiogrammopsis minima* belongs to the epipsammon of sandy sediments where it lives attached to sand grains, either solitary or in short, zig-zag colonies. In the Westerschelde estuary, the species is restricted to fine and medium sandy sediments in the poly- to euhaline reaches, where it is most abundant in summer. *P. minima* has been reported from sandy sediments in the North Sea area and the Portuguese Atlantic coast (Salah 1955, Colijn & Nienhuis 1978, as *Plagiogramma* sp. 1, Vos 1986, Denys 1991, this study) and from North America (Cooper 1995, Witkowski *et al.* 2000).

Plagiogrammopsis sigmoidea (Salah 1955) Sabbe & Muylaert comb. nov. (Figs. 2-6)

Basionym: *Plagiogramma sigmoideum* Salah 1955, Hydrobiologia 7, p. 90, Pl. I, Figs. 10-13.
Lectotype: Blakeney 5B, 1949, BM 36399, design. Sabbe K.
Synonym: *Plagiogrammopsis mediaequatus* Gardner & Crawford 1994
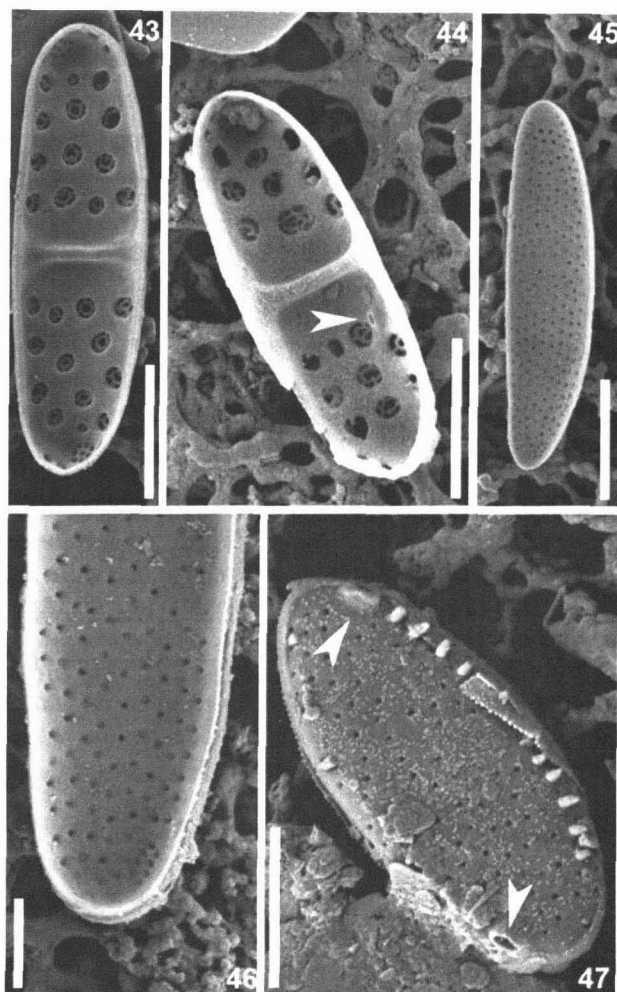
Figs 43-47. – *Plagiogrammopsis minima* and *Pierrecomperia catenuloides*, SEM, samples from the Tagus estuary (Portugal). Scale bars represent 1 μm (Fig. 46), 2 μm (Figs 43-44, 47) or 3 μm (Fig. 46). Figs 43-44. *Plagiogrammopsis minima*. Internal valve view showing the central submarginal process (arrow) in Fig. 44. Fig. 45-47. *Pierrecomperia catenuloides*. Fig. 45-46. Internal valve views. Note diagonally opposed ocelluli in Fig. 45 and areolar occlusions (vela) in Fig. 46. Fig. 47. External valve view with arrowheads indicating the ocelluli.

Comment: the cells of *P. sigmoidea* are rectangular in girdle view, about 6.9 μm wide, and show distinctly raised apices and a conspicuous, convex central fascia (Fig. 2). Plastids were not observed. The valves are lanceolate with slightly produced to rostrate apices. They are sometimes slightly sigmoid (Figs. 4, 6). In valve view, the central fascia appears to be round. The areolae are arranged in longitudinal rows, parallel to the valve margins.

A detailed description of this species was given by Gardner & Crawford (1994, as *Plagiogrammopsis mediaequatus*), where it is shown that *P. sigmoidea* belongs to the genus *Plagiogrammopsis*. Although we have not been able to obtain SEM images of this species in our own material, there is no doubt that *P. sigmoidea* is conspecific with *P. mediaequatus*: they are identical in LM and have the same dimensions and stria density (see also Witkows-
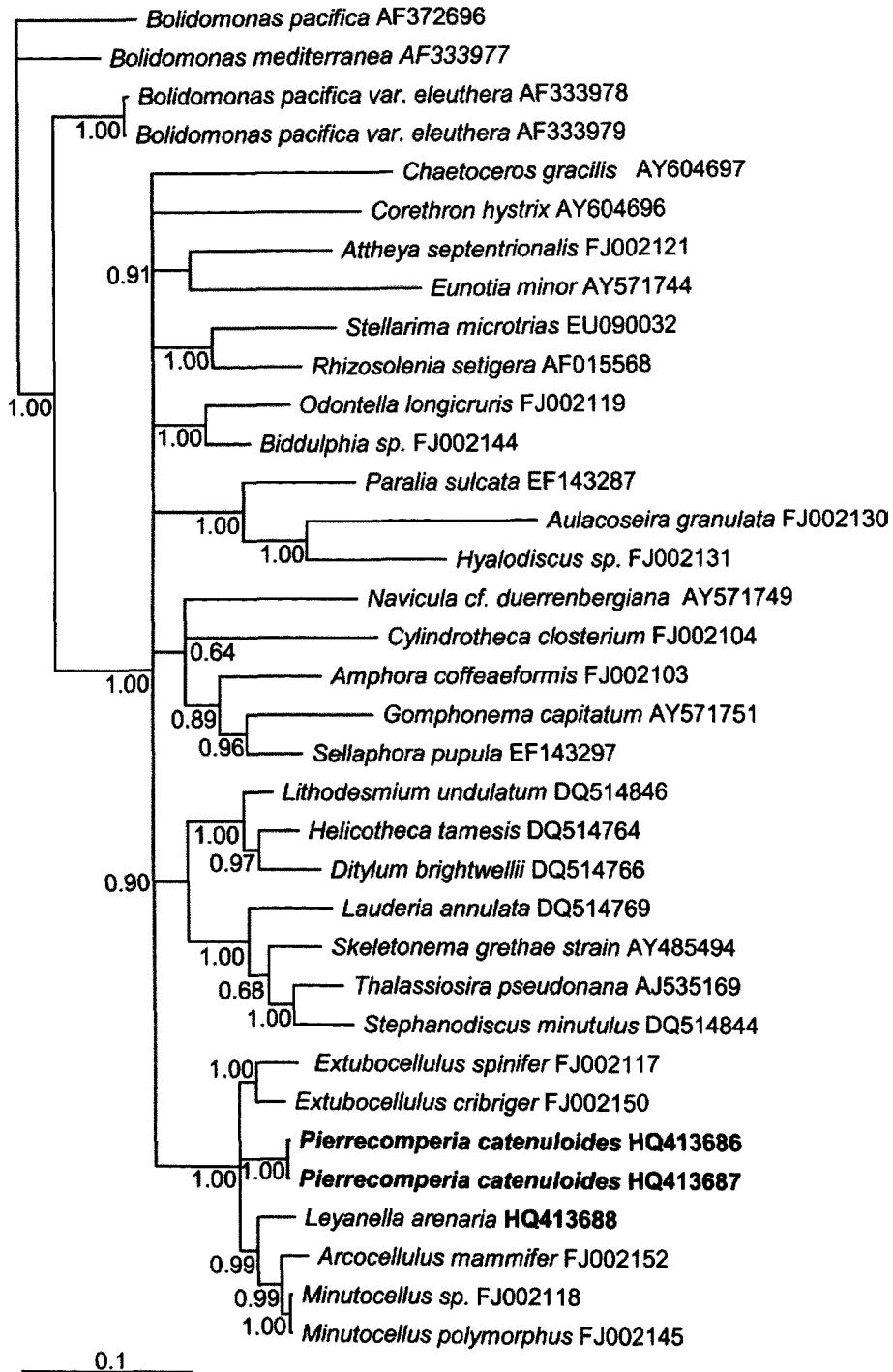
Fig. 48. – Phylogeny obtained from Bayesian inference of the *rbc*L gene. The tree shown is the 50 % majority-rule consensus tree based on BI analysis. Posterior probabilities > 0.7 are indicated on the respective nodes. Scale bar represents one substitution in ten nucleotides.

ki *et al.* 2000, Pl. 10, Figs. 26-30). *P. sigmoidea* differs from *P. vanheurckii* in the absence of a pseudoseptum, the presence of a wing on the pili (not observed in this study) and the less pronounced apical elevations (cf. Fig. 2) (Gardner & Crawford 1994). Despite these differences however, we agree with Gardner & Crawford (1994) that *P. sigmoidea* should be placed in the genus *Plagiogram-mopsis*.

Biogeography and ecology: *P. sigmoidea* was described from intertidal sediments at Blakeney Point (England). Gardner & Crawford (1994) observed it (as the synonym *P. mediaequatus*) in intertidal sediment and inshore plankton samples collected at various locali-ties around the British Isles. The specimens illustrated in Witkowski *et al.* (2000) are from the Azores and the USA (Mississippi Delta). It has also been reported from Holo-

cene deposits of the Belgian coastal plain (Denys 1991). In the Westerschelde, it was common in silty sediments in the middle and lower reaches of the estuary.

Subfamily Extubocelluloidea Hasle, von Stosch & Syvertsen

Pierrecomperia Sabbe, Vyverman & Ribeiro gen. nov.
(Figs. 21-27, 37-38, 40-42, 45-47)

Descriptio : cellulae sine processibus, facie connectivali rectangulares, formantes colonias taeniformes. Valvae semi-ellipticae ad plus minusve semi-arcuatae, sine sternis vel fascia. Unus plastidus ad valvam appressus. Frons valvae plus minusve plana ad curvata in sectione transapicali ; pars centralis valvae nonnumquam elevata. Limbus non profundus. Areolae minutae, plus minusve irregulariter distributae. Junctura frontis cum limbo spinifera ; spinae simplices, nonnumquam bifurcatae. Ocelluli subapicales diagonaliter oppositae. Cingulum profundus, ex copulis apertis, subtiliter porosis, leviter ligulatis constans. Habitat in mari, probabiliter ad floram interstitialem vel affixam sabuletorum.

Description: cells without processes, rectangular in girdle view, forming ribbon-like colonies. Valves semi-elliptical to slightly semi-arcuate; no sternum or fascia is present. One plastid per cell, lying beneath one valve. Valve face more or less flat to curved (in transapical direction); sometimes, the central part appears to be raised (Fig. 41). Mantle shallow. Areolae very small, more or less irregularly distributed. Simple, sometimes bifurcate linking spines are present along the valve face margin. Subapical ocelluli diagonally opposite. Cingulum deep, copulae numerous, finely porous, open, slightly ligulate. Probably belongs to the interstitial or attached flora of marine sand flats.

Typus generis: *Pierrecomperia catenuloides* Sabbe, Vyverman & Ribeiro sp. nov.

Etymology: named in honour of the Belgian phycologist Pierre Compère of the National Botanical Garden (Meise, Belgium) for his continuous support and generous assistance with taxonomic and nomenclatural problems.

Pierrecomperia catenuloides Sabbe, Vyverman & Ribeiro sp. nov. (Figs. 21-27, 37-38, 40-42, 45-47)

Descriptio : cellulae facie connectivali rectangulares, 2.3-4.1 μm latae, formantes colonias taeniformes. Unus plastidus ad valvam appressus. Valvae semi-ellipticae ad plus minusve semi-arcuatae, sine sternis vel fascia, 5.7-15.6 (11.2 ± 3.0) μm longae, 2.2-3.5 (2.7 ± 0.4) μm latae. Frons valvae plus minusve plana ad curvata in sectione transapicali; pars centralis valvae nonnumquam elevata. Limbus non profundus. Areolae minutae, plus minusve irregulariter distributae, 23-29 in 10 μm. Junctura fron-

tis cum limbo spinifera ; spinae simplices, nonnumquam bifurcatae. Ocelluli subapicales diagonaliter oppositae. Cingulum profundus ex quatuor copulis apertis, subtiliter porosis, leviter ligulatis constans.

Description: cells rectangular in girdle view (2.3-4.1 μm wide), forming ribbon-like colonies. One plastid per cell, lying beneath one valve. Valves semi-elliptical to slightly semi-arcuate with cuneate to rostrate apices, 5.7-15.6 (11.2 ± 3.0) μm long, 2.2-3.5 (2.7 ± 0.4) μm wide. No sternum or fascia is present. Valve face more or less flat to curved (in transapical direction); sometimes, the central part appears to be raised. Mantle shallow. Areolae very small, irregularly distributed across the valve surface, ± 23-29 in 10 μm. Simple, sometimes bifurcate linking spines are present along the valve face margin. Subapical ocelluli diagonally opposite. Cingulum deep, copulae 4, open, finely porous, slightly ligulate.

Holotype: BM slide 101464, The Natural History Museum, Department of Botany, London (BM)

Isotype: slide KS1002, The Herbarium, Ghent University (GENT).

Type locality: Sandy beach (median grain size 305 μm) at Vlissingen (Ritthem) near the mouth of the Westerschelde estuary (The Netherlands); salinity range 27.5-33 ‰ (30.3 ± 2.5).

Etymology: this specific epithet refers to the resemblance of this species to *Catenula adhaerens* Mereschkowsky in LM.

Comment: the cells of *Pierrecomperia catenuloides* typically form ribbon-like colonies of up to at least 8 cells and are usually closely associated with detrital particles (Figs. 21-23). There is one plastid per cell (Fig. 22) which appears to be appressed against one valve (Fig. 23). The frustules are rectangular in girdle view, the transapical axis is about 2.3-4.1 μm (Figs. 21, 23). The valves are more or less semi-elliptical to distinctly asymmetrical about the apical plane (Figs. 22, 24-27, 45, 47); the apices are cuneate to rostrate. The valve face is more or less flat; the mantle is shallow and has no areolae. Sometimes the centre is more or less bulged (Fig. 41) as in *Brockmanniella brockmannii* (Fig. 39, cf. also Hasle *et al.* 1983). Externally, a ring of simple interlocking spines is present on the margin of the valve face but not on the apices (Fig. 37, 41, 47). The valve face is perforated by numerous scattered, tiny poroids, which are arranged randomly or in short, irregular rows. They are occluded by vela (Fig. 46) but these are usually eroded (Figs 37, 38, 40). At each pole a raised ocellulus is present; externally, they are surrounded by a distinct rim (Figs. 37, 40, 47). They consist of a ring of porelli (Figs. 45, 46), often surrounding one or two central porelli (Figs. 37, 38). The ocelluli are diagonally opposed to one another in a clockwise direction (Figs. 38, 45, 47). No processes or hyaline areas (such as a central sternum or a fascia) are present, so the cells are isovalvate. We found no evidence of specialized end valves. The cingulum appears to consist of 4 open,
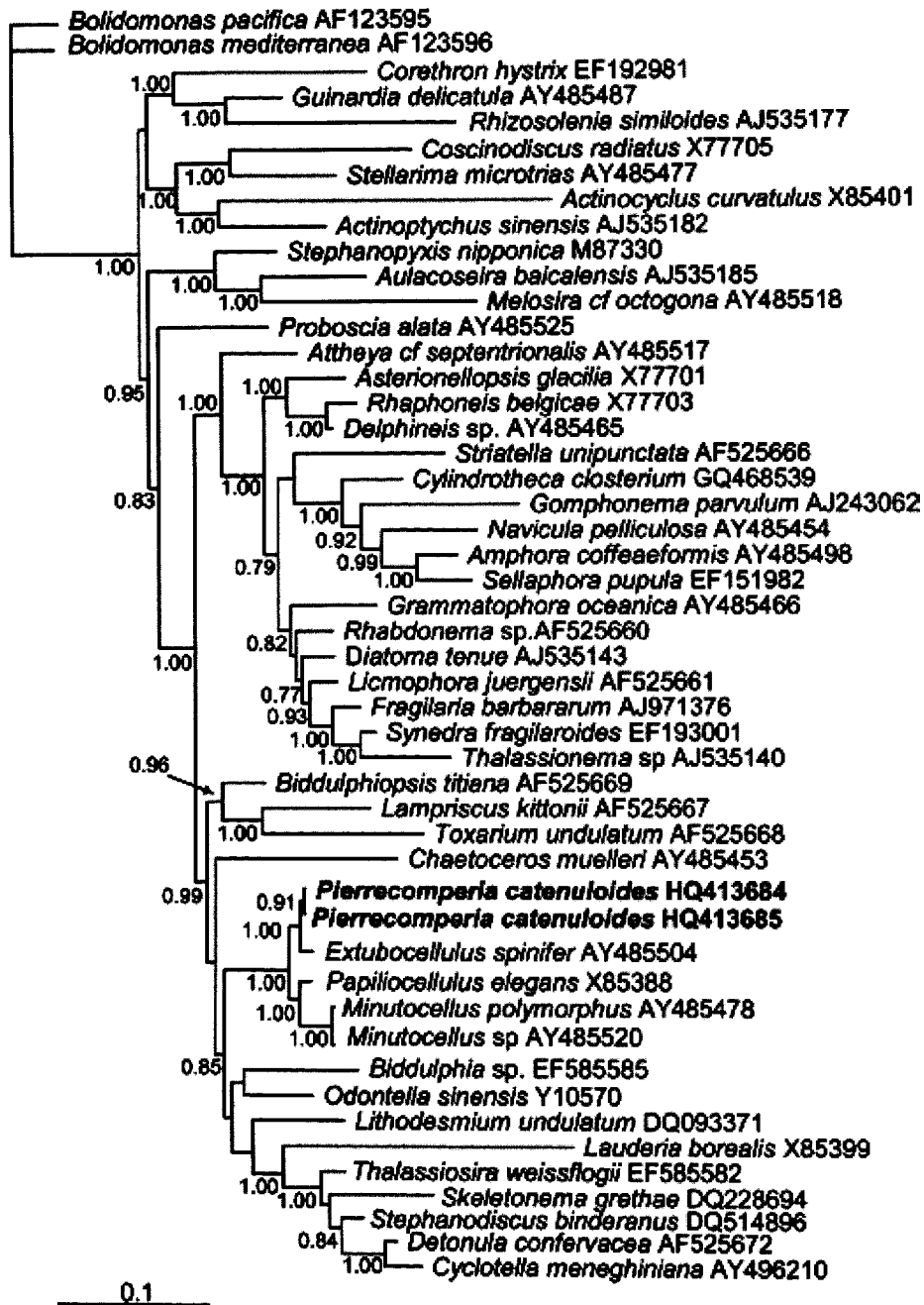
Fig. 49. – Phylogeny obtained from Bayesian inference of the 18S rRNA gene. The tree shown is the 50 % majority-rule consensus tree based on BI analysis. Posterior probabilities > 0.7 are indicated on the respective nodes. Scale bar represents one substitution in ten nucleotides.

slightly ligulate copulae (Fig. 40). In at least one frustule longitudinal rows of very fine perforations can be seen on the copulae (Fig. 37).

In LM, *Pierrecomperia catenuloides* can easily be confused with *Catenula adhaerens*. This species has a similar shape and dimensions but is more robust and has no scattered areolae but (indistinct) striae (see Sundbäck and Medlin 1987 for description and illustrations). It also forms ribbon-like colonies and occurs in the same, psammic habitat. However, in SEM it becomes clear that *Catenula adhaerens* is a completely different, biraphid

diatom which is probably closely related to *Amphora* Ehrenberg (Round *et al.* 1990). *Pierrecomperia catenuloides* also strongly resembles *Campylosira inane* Giffen, a species which was described by Giffen (1975) from South African coastal waters. As for *Cymatosira capensis* (cf. above) it is hard to assess the true identity of the latter species without a thorough study of the type material. However, from Giffen's description it appears that *Campylosira inane* is a slightly larger and more robust diatom than *Pierrecomperia catenuloides*. *Campylosira inane* almost certainly does not belong to the genus

Campylosira; it might belong to *Pierrecomperia*.

Analyses of the *rbc*L and 18S rRNA gene firmly place *Pierrecomperia* in the Cymatosiraceae clade (Figs. 48, 49). While its position within this clade is unresolved in the *rbc*L tree, the 18S rRNA suggests that *Pierrecomperia* is sister to the *Extubocellulus* clade. Note also that the monophyly of the Cymatosiraceae is completely supported (posterior probability = 1) in both trees.

Biogeography and ecology: *Pierrecomperia catenuloides* was rather common in fine and medium sandy sediments in the poly- and euhaline reaches of the Westerschelde estuary. In the Tagus estuary, it was rare (up to 1.1 %) in the same habitat. Unlike *Cymatosira belgica* and *Brockmaniella brockmannii* which also form ribbon-like colonies it was usually not found in silty sediments. We also found valves belonging to this taxon in Hustedt's material from the German Wadden Sea (Hustedt 1939, BRM slide N12/36, Leybucht, Leysand, Fig. 25); it probably occurs throughout the North Sea area.

DISCUSSION

On the basis of morphological and molecular-phylogenetic data we propose *Pierrecomperia* as a new genus in the centric diatom family Cymatosiraceae (Hasle *et al*. 1983). Like the other genera in this family, *Pierrecomperia* is bipolar but completely lacks a pennate valve face pattern. In addition, it has two low polar elevations per valve, each with an ocellulus, which is hitherto only known from the Cymatosiraceae. As the other members of this family, *Pierrecomperia* possesses a single, plate-like plastid [cf. plastid structure in e.g. *Cymatosira belgica* (Fig. 1), *Plagiogrammopsis minima* (Fig. 7) and *Leyanella arenaria* (Fig. 14)]. Molecular-phylogenetic analyses based on the *rbc*L and 18S rRNA genes firmly place *Pierrecomperia* in the Cymatosiraceae.

Pierrecomperia is strikingly similar to the genus *Nephroneis*, also described from the intertidal zone of a marine, sandy beach (Amspoker 1989). The latter genus however does not possess ocelluli and has one (or two) subapical rimoportulae and a pennate valve face pattern (with scattered areolae in the centre of the valve face but distinct transapical rows of areolae throughout the rest of the valve face and mantle). This combination of features plus the absence of certain features typical of other centric genera (such as a marginal ring of processes) led Amspoker (1989) to allocate *Nephroneis* to the Fragilariophyceae.

Pierrecomperia resembles *Extubocellulus* in the absence of pili or marginal ridges (Table I). However, *Extubocellulus* differs from *Pierrecomperia* in valve and cell shape (the pervalvar axis is usually longer than the apical one in *Extubocellulus*), the absence of marginal spines, and the presence of quasifract bands (note however that the latter are not always present, e.g. Riaux-Gobin

& Chrétiennot-Dinet 2000). Processes have only rarely been observed in *Extubocellulus*; they are absent in *Pierrecomperia*.

Finally, *Pierrecomperia* superficially looks like *Brockmanniella*, which also has rather flat cells with a slightly convex centre bordered by shallow depressions, two offset, laterally opening ocelluli and a marginal ring of spines (Fig. 39). Like *Pierrecomperia*, *Brockmanniella* has no pili or pseudoseptum. However, *Pierrecomperia* differs from *Brockmanniella* in the absence of a fascia, the structure of the spines and the areolae, and the absence of subcentral processes.

Pierrecomperia is isovalvate: it has no pili or processes and probably does not possess specialized end valves. Hasle *et al*. (1983) established the subfamily Extubocelluloidea to accommodate the only isovalvate genus known at that time, namely *Extubocellulus*. Takano (1985) described a second isovalvate genus *Pseudoleyanella* and placed it in the Extubocelluloideae. *Pierrecomperia* is the third isovalvate genus in the Cymatosiraceae and should also be assigned to the Extubocelluloideae according to the original description (Hasle *et al*. 1983). This allocation is confirmed by the 18S rRNA analyses in which *Pierrecomperia* is sister to the *Extubocellulus* clade. The absence of a tubular process is probably a derived feature in the Cymatosiraceae: Hasle *et al*. (1983) point out that the occasional presence of tubular processes in *Extubocellulus* may be indicative of former heterovalvy.

Many Cymatosiraceae exhibit extreme stadial variation, expressed in changes in size, shape, cingulum structure (e.g. quasifraction), valve curvature, areolation pattern and loss of certain structures (such as pili, spinulose areas and marginal ridges) in minimum valves (i.e. valves from the lowest end of the size spectrum). This variation causes cells belonging to opposite ends of the size spectrum to be morphologically dissimilar (Hasle *et al*. 1983). *Cymatosira minutissima* and *Plagiogrammopsis minima* are quite reminiscent of the minimum valves of *Cymatosira belgica* and *Plagiogrammopsis vanheurckii* [as illustrated in Hasle *et al*. (1983) Fig. 118], respectively, as they lack some features that are characteristic of the genera they belong to (e.g. well-developed spines in *C. minutissima* or pili in *P. minima*). However, on the basis of morphological, ecological and physiological grounds it is in both cases highly unlikely that they actually do concern minimum valves of these existing species: (1) *Cymatosira minutissima* consistently has a much higher areolar density than *C. belgica*, namely 19-24 versus 9-12 in 10 μm (measured in 20 Westerschelde specimens) respectively, a feature which is normally rather constant within species (Round *et al*. 1990). *Plagiogrammopsis minima* always (i.e. even in large cells, e.g. Fig. 10) has a distinctly simpler valve shape and structure than *P. vanheurckii* (see Hasle *et al*. 1983) the valves are not (or only slightly) constricted near the apices and are only slightly convex in the centre. (2) There is a high

Table I. – Comparison between main morphological features of *Pierrecomperia* gen. nov. and other similar genera belonging to the diatom family Cymatosiraceae (– = absent, + = present, +/– = present or absent).

	Pierrecomperia	Brockmanniella	Cymatosira	Plagiogrammopsis	Extubocellulus	Pseudoleyanella
Fascia	–	+	–	+	–	–
Pseudoseptum	–	–	–	+/–	–	–
Pili	–	–	–	Incomplete	–	–
Marginal spines	Short linking spines	Long spines, colonies separable	Spines, usually interlocking	Long spines, colonies separable	Short spines on valve face and margin, or absent	
Ocelluli	Two, offset, opening laterally	Two, offset, opening laterally	Two, opening only slightly laterally	Two, opening laterally	Two, opening only slightly laterally	Two, offset, different shape
Process	–	One rimoportula/cell, central, submarginal	One rimoportula/cell, central, submarginal	One rimoportula/cell, central, submarginal	Rare	–
Specialized end valves	–	–	+	–	–	
Occlusion type	Simple poroids occluded by vela	External cribrum; no spinules (?)	External cribrum with spinules	External cribrum with spinules	Simple external cribrum or poroids	External cribrum
Cingulum	Numerous (8) porous copulae	10-15?, ligulate, porous copulae	4-7 open, porous bands	5-8 split, ligulate, porous copulae	Numerous, porous and/or quasifract	Numerous?
Other	Isovalvy	Isovalvy		Spindle-shaped foramen between cells	Isovalvy	Isovalvy

degree of ecological segregation between populations of *C. minutissima* and *P. minima* on the one hand (which are typical of more coarsely grained sandy sediments) and *C. belgica* and *P. vanheurckii* on the other (which occur in silty sediments or in the plankton, Muylaert & Sabbe 1999). Although there are indications that physiological and hence possibly ecological differentiation can occur between smaller and larger cells belonging to one clone (or population) (Werner 1971, Paasche 1973, Round *et al.* 1990), it seems very unlikely that such a high degree of niche differentiation and spatial segregation would be found amongst cells belonging to the same cell size reduction sequence. (3) The fact that thriving populations of both *C. minutissima* and *P. minima* were present cannot be reconciled with published evidence which shows that at least in some species minimum cells are less vital than larger cells (for example because of lower growth rates, Paasche 1973).

However, we do believe that by analogy with the loss of characters with diminishing size during the cell size reduction cycle, the apparent simplicity of the valves of *Cymatosira minutissima* and *Plagiogrammopsis minima* could be attributed to their overall smaller size in comparison with other members of these genera.

The findings of the present study indicate that the current low number of species in the Cymatosiraceae may be mainly due to the general lack of studies on the small-sized diatom flora of marine sandy sediments (Gardner & Crawford 1994, Sabbe & Vyverman 1995, Sabbe *et al.* 1999). Most taxa belonging to the Cymatosiraceae have been described from and are usually found in sediments although they also appear in the plankton of shallow seas and estuaries (Hasle *et al.* 1983). However, despite their widespread and common occurrence little is known about their exact life-forms and life strategies. In the Westerschelde estuary, *Brockmanniella brockmannii*, *Campylosira cymbelliformis*, *Cymatosira belgica*, *Plagiogrammopsis sigmoidea* and *P. vanheurckii* (which all form ribbon-like colonies) were most abundant in silty sediments which are dominated by epipelic (mainly *Navicula* spp.) and other free-living species [such as *Delphineis minutissima* (Hustedt) Simonsen and *Thalassiosira proschkinae* Makarova]. However, whether these five species actually prefer a benthic or pelagic habitat in nature or whether they successfully exploit both habitats and thus adopt a truly tychoplanktonic life style is as yet not well known (e.g. for *Cymatosira belgica* see Sabbe 1993 and Denys 1995). As in many other supposedly tychoplanktonic taxa [such as *Thalassiosira proschkinae* and *T. decipiens* (Grunow) Jørgensen, cf. Muylaert & Sabbe 1996] their cells are often associated with sediment and detritus particles (cf. also Hasle *et al.* 1983), which could accelerate sinking from the water column after resuspension. A tychoplanktonic life-form might then enable these diatoms to fully exploit both the benthic and pelagic environments, by commuting between the water column (with

more favourable light conditions than subtidal sediments) and sediments (where nutrients are often more abundant) in shallow, subtidal environments or enabling them to prolong their stay in the intertidal zone, where both light climate and nutrient conditions are favourable at low tide.

Pierrecomperia catenuloides, *Cymatosira minutissima*, *Leyanella arenaria* and *Plagiogrammopsis minima* were predominantly found in sandy, more dynamic sediments. Their absence from silty sediments and the water column (Muylaert & Sabbe 1999) excludes a tychoplanktonic life style for these species. Only the latter species however can with certainty be characterized as an epipsammic species. The exact life-form of the others is unknown; possibly they live loosely attached to sand grains or simply lie on the sediment as *Stoschiella hebetata* Gardner & Wenderoth (Gardner *et al.* 1995).

Little is known about the biogeography of most taxa of the Cymatosiracae. Only the more robust (and hence longest known) species *Campylosira cymbelliformis*, *Cymatosira belgica* and *Plagiogrammopsis vanheurckii* have been reported worldwide (Frenguelli 1938, Hendey 1964, Giffen 1971, Foged 1975, McIntire & Overton 1971, Navarro 1982, Hasle *et al.* 1983, Kosugi 1987, Podzorski & Håkansson 1987, Laws 1988, Garcia-Baptista 1993, Cooper 1995, Hemphill-Haley 1995, Muylaert & Sabbe 1999) Whether the other species have a more restricted geographical distribution is as yet impossible to assess although it seems more likely that due to their fragile nature or minute size they have been overlooked in most studies.

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