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Similar morphological and chemical variations of *Gloeocapsomorpha prisca* in Ordovician sediments and cultured *Botryococcus braunii* as a response to changes in salinity

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Abstract—Most Ordovician source rocks consist of accumulations of a colonial marine microorganism, *Gloeocapsomorpha prisca* (*G. prisca*) whose nature, ecology and affinity with extant organisms have been in dispute for years. Furthermore, recent studies have shown major differences in phenol moieties between two *G. prisca*-rich samples. Examination of five *G. prisca*-rich kerogens by electron microscopy and pyrolysis studies revealed (i) the occurrence of two markedly distinct "morpho/chemical" types: a "closed/phenol-rich" type (Baltic samples) and an "open/phenol-poor" one (North American samples) and (ii) the selective preservation of the resistant macromolecular material building up the thick cell walls in the original organism. Comparison with extant *Botryococcus braunii* (a widespread green microalga) grown on media of increasing salinity suggests that *G. prisca* is likely to be a planktonic green microalga related to *B. braunii*, which can adapt to large salinity variations which, in turn, control its polymorphism. The large differences in colony morphology and in the content of phenol moieties observed in fossil *G. prisca* and the resulting occurrence of two "morpho/chemical" types, should therefore reflect depositional environments with different salinities. The presence of thick, highly aliphatic, resistant walls in *G. prisca* selectively preserved during fossilization, accounts for the major contribution of this organism to Ordovician organic-rich sediments and for the resulting typical signature of Ordovician oils

Key words—*Gloeocapsomorpha prisca*, *Botryococcus braunii*, kukersites, Ordovician source rocks, flash pyrolysis, scanning electron microscopy, phenol moieties, selective preservation, salinity influence

INTRODUCTION

Studies by light or u.v. fluorescence microscopy have revealed that a significant number of Ordovician kerogens consist almost entirely of accumulations of the fossil remains of a marine microorganism, called *Gloeocapsomorpha prisca*. The widespread occurrence of organic-rich, *G. prisca*-dominated Ordovician sediments in North America, Australia and Northern Europe reflects the huge growth of this colonial organism in tropical epicontinental seas during the Ordovician (reviewed by Fowler, 1991). For example, the Estonian *G. prisca*-rich deposits (Baltic basin) cover an area over 50,000 km^2 with a cumulative thickness up to 20 m (Lindenbeim, 1921; Baukov, 1973). These Estonian oil shales have been extensively mined and the present production is 25–30 million tons per year (Foster *et al.*, 1989a).

The possible occurrence of *G. prisca* in Precambrian and Silurian sequences has been reported by Cramer and Diez de Cramer (1972) and by Konzalova (1973) whilst more recent studies suggest that some Cambrian deposits consist of accumulations of a related microorganism (Wielens *et al.*, 1990; Fowler, 1991).

Analyses of both bitumens and kerogen pyrolysates of various *G. prisca*-rich Ordovician deposits revealed unusual distributions of saturated hydrocarbons i.e. a dominance of odd-carbon-numbered *n*-alkanes in the C_9–C_{19} range, relatively low amounts of C_{20+} *n*-alkanes, substantial levels of *n*-alkylcyclohexanes, relatively very low contributions of acyclic isoprenoid hydrocarbons (Klesment, 1974; Klesment and Nappa, 1980; Fowler, 1984; Fowler *et al.*, 1986; Reed *et al.*, 1986; Hoffmann *et al.*, 1987; Longman and Palmer, 1987; Jacobson *et al.*, 1988; Fowler, 1991; Douglas *et al.*, 1991a, b). Similar alkane distributions were observed in numerous Ordovician oils from various basins but not in oils of a different age (Martin *et al.*, 1963; Powell and McKirdy, 1972; Longman and Palmer, 1987; Hatch *et al.*, 1987; Derenne *et al.*, 1990 and ref. therein). In fact, as determined by correlation studies (Fowler *et al.*, 1986; Reed *et al.*, 1986; Hoffmann *et al.*, 1987), *G. prisca* was a major contributor to kerogens sourcing most Ordovician oils. In spite of extensive studies which mainly focused on the analysis of associated bitumens, the nature of *G. prisca*, its ecology and its possible affinities with extant microorganisms are still far from being

understood (Fowler, 1991). *G. prisca* has been considered a cyanobacterium (Zalessky, 1917; Tynni, 1975; Klesment and Nappa, 1980; Burns, 1982; Foster *et al.*, 1989b, 1990; Stasiuk and Osadetz, 1990), an extinct green alga (Fowler and Douglas, 1984; Douglas *et al.*, 1991a, b), a *Botryococcus*-type alga (Lindenbeim, 1921; Traverse, 1955; Konzalova, 1973; Monin *et al.*, 1980; Glikson *et al.*, 1989), a planktonic and photosynthetic organism, possibly an eukaryotic lipid-rich alga (Hoffmann *et al.*, 1987) and a non-photosynthetic, mat-forming, prokaryotic organism (Reed *et al.*, 1986). Furthermore, recent studies of two *G. prisca*-rich Ordovician kerogens raised further questions concerning the chemical structure and origin of these fossil remains (Derenne *et al.*, 1990). Several pyrolysis techniques in combination with GC–MS analysis revealed that *ca* 60% of the pyrolysate of an Estonian kukersite consists of phenolic compounds. The data indicated, for the first time, that abundant phenol moieties can be present in marine Type II/I kerogens without any contribution of higher plants. Series of non-methylated and mono-, di- and trimethylated 3-*n*-alkylphenols, 5-*n*-alkyl-1,3-benzenediols as well as tentatively identified *n*-alkylhydroxybenzofurans were observed (Derenne *et al.*, 1990). In sharp contrast, no alkylphenols could be detected in the flash pyrolysate of a sample of the Guttenberg Oil Rock (GOR; S.W. Wis., U.S.A.; Douglas *et al.*, 1991a, b) although both the Estonian and the Wisconsin kerogens almost entirely consist of *G. prisca* colonies, as revealed by light or u.v. fluorescence microscopy, and showed similarities in hydrocarbon and sulphur compound distributions in their pyrolysates.

The main purpose of the present study was to investigate the origin of the phenol moieties in *G. prisca*-rich kerogens and to rationalize the major quantitative differences of such moieties in these kerogens. To this end five *G. prisca* kerogens from Northern Europe and North America, including the aforementioned samples from Estonia (K1) and from Wisconsin (GOR), were compared with regard to both their ultrastructural features, revealed by electron microscopy, and their pyrolysis products.

EXPERIMENTAL

The procedures for kerogen and bitumen isolation (Derenne *et al.*, 1990; Douglas *et al.*, 1991a), transmission electron microscopy (TEM) and scanning electron microscopy (Largeau *et al.*, 1990) were as described previously.

PY–GC and PY–GC–MS: For analysis by pyrolysis–gas chromatography (PY–GC) and pyrolysis–gas chromatography–mass spectrometry (PY–GC–MS) samples were applied to a wire with a Curie-temperature of 610°C and flash-pyrolysed with a FOM-3LX Curie point pyrolyser. Pyrolysis products were trapped cryogenically and subsequently chromatographed. Mass spectra were recorded with a VG 70 S mass spectrometer operated at 70 eV. "Off-line" pyrolysis was carried out at 400°C under a flow of He; the pyrolysate was trapped in $CHCl_3$ at −5°C and separated by column chromatography into three fractions eluted with hexane, toluene and methanol, respectively. Identification of the constituents of these fractions was carried out by GC–MS with a Nermag-R10-10 mass spectrometer operated at 70 eV (Derenne *et al.*, 1990). *B. braunii* was grown under air-lift conditions on a modified CHU 13 medium (Casadevall *et al.*, 1985). Isolation of the non-hydrolysable macromolecular constituent (PRB) of *B. braunii* outer walls: briefly, the dry biomass was successively extracted with hexane and $CHCl_3$/MeOH (2/1, v/v), saponified by refluxing for 6 h in 6% KOH in MeOH/H_2O (9/1, v/v) and finally treated by concentrated H_3PO_4 for 13 days at 55°C; extensive extractions by H_2O, EtOH, Me_2CO and Et_2O were carried out after the above basic and acid treatments (Berkaloff *et al.*, 1983).

RESULTS AND DISCUSSION

Morphology and chemistry of two distinct types of G. prisca

TEM and SEM studies revealed identical morphologies of the kerogens from the Baltic basins, i.e. two Estonian kukersites (K1 and K2) and that from the Weimarn deposit near Leningrad (K3). The *G. prisca* colonies, accounting for the bulk of these kerogens, are composed of tightly packed cells. SEM [Plate 1(A–D)] and TEM (Plate 2) observations revealed cell voids completely ensheathed by thick (*ca* 1.5 μm) multilamellate walls and SEM indicated that cell voids do not open to the surface of the colonies. The cell contents have obviously been degraded entirely during fossilization whereas the thick cell walls were perfectly preserved during diagenesis. This is consistent with previous assumptions regarding the fossilization of *G. prisca*, i.e. the selective preservation of a resistant macromolecular constituent originally present in the cell walls of this organism (Derenne *et al.*, 1990; Douglas *et al.*, 1991a, b).

Examination by electron microscopy also showed similar morphologies of colonies in the two *G. prisca*-rich Ordovician samples from North America, GOR (Wis., U.S.A.) and K91 (Ontario, Canada). These observations indicated a selective preservation of thick resistant outer as well as a complete elimination of the cell contents [Plate 1(E,F)]. However, in sharp contrast with the Baltic kerogens, the cells are not entirely surrounded by a thick resistant wall and individual cell voids open to the surface of the colonies. In the case of GOR and K91, only the basal part of cells was embedded in a thick "cup-like" resistant wall. Electron microscopy therefore revealed clear differences in the general organization of *G. prisca* colonies of the Baltic and the North American samples. We define these two markedly

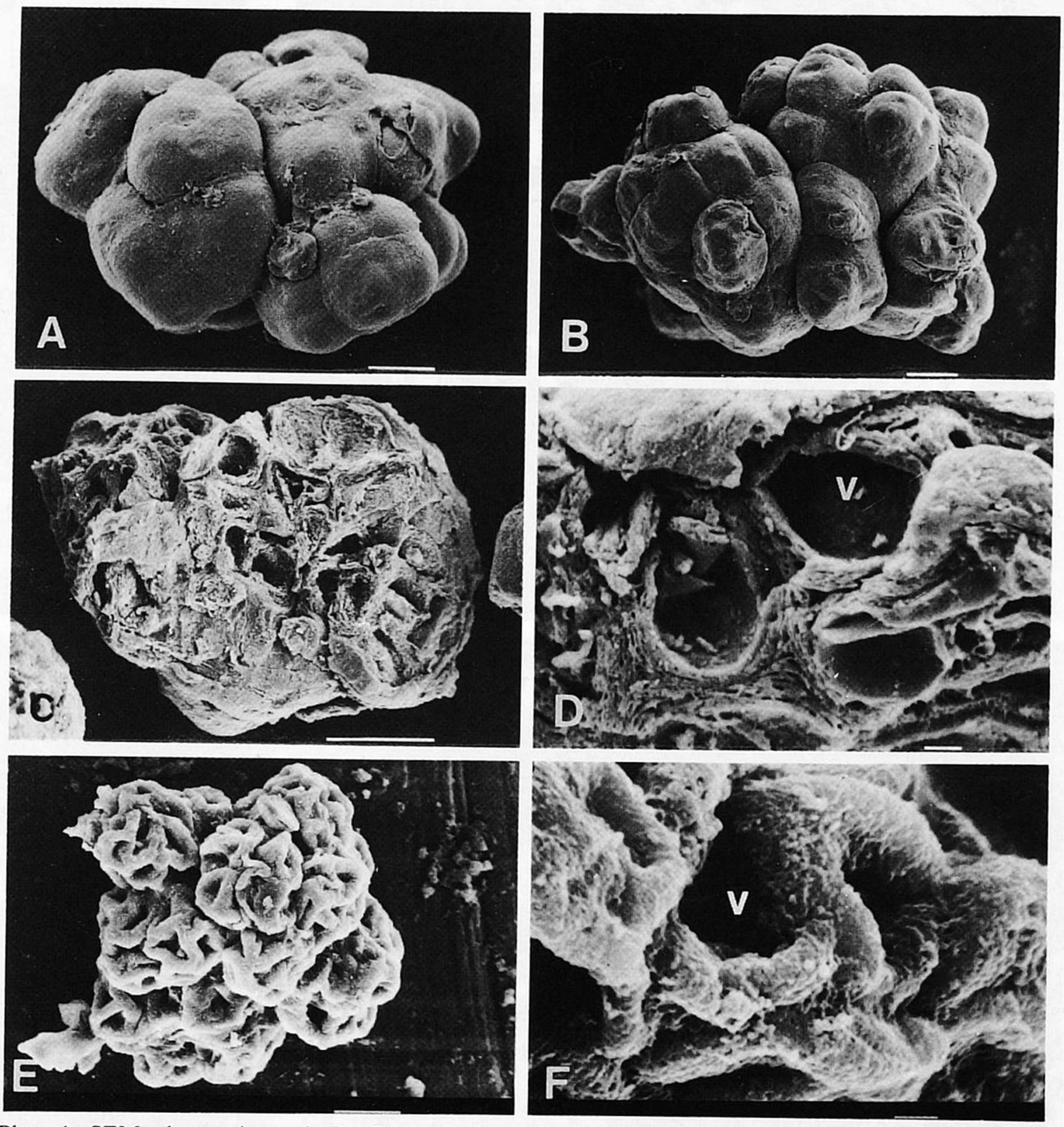
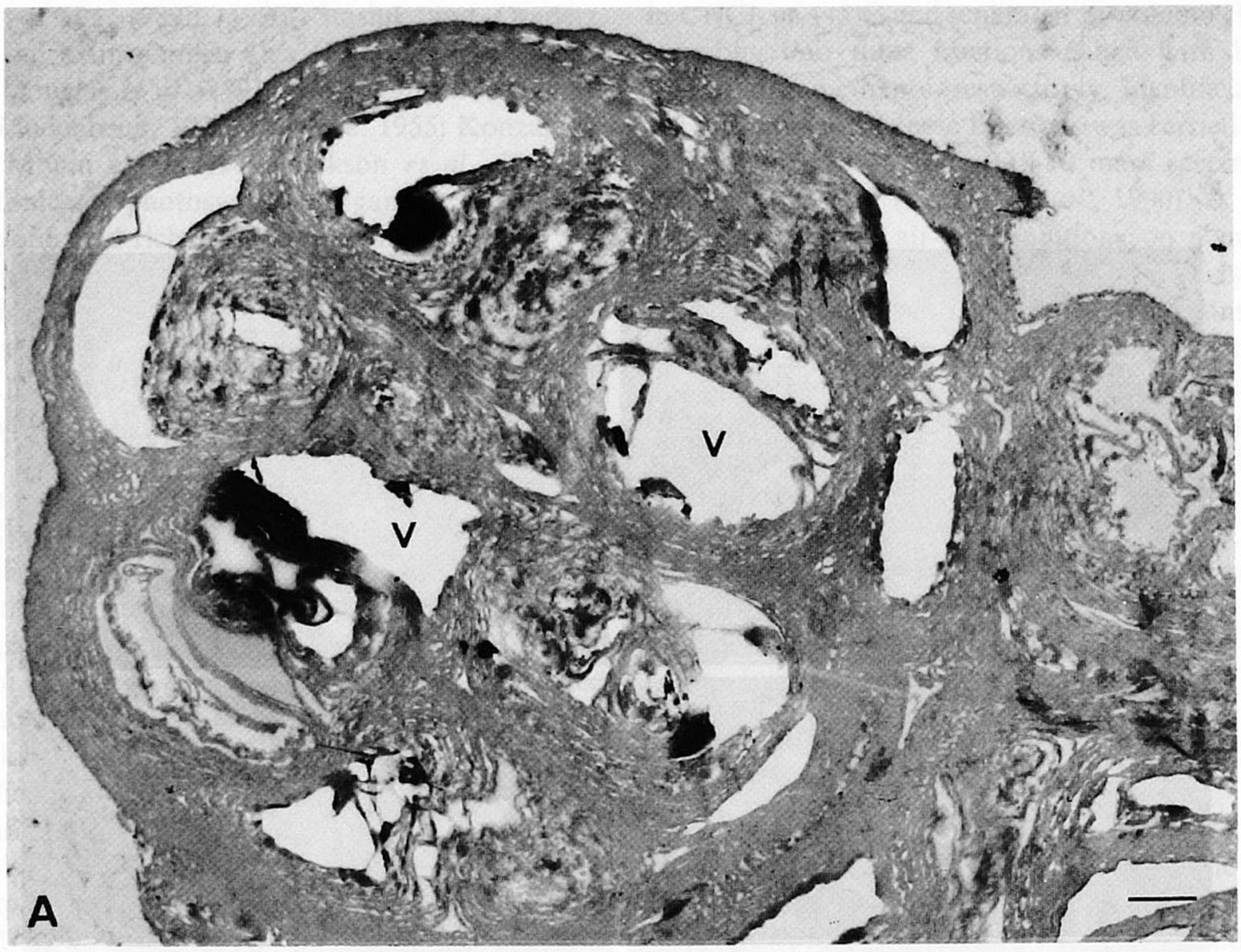


Plate 1. SEM observations; A–D, *G. prisca* from the Baltic basin ("Closed wall" type) K1 sample. A (×1400) and B (×1100): examination of whole colonies reveals a smooth surface and an excellent preservation of their general morphology. C (×2300) and D (×8000): cross-sections of colonies. The presence of numerous cell voids (**v**), surrounded by thick lamellate walls, is noted. (Identical morphological features were observed from K2 and K3 kerogens.) E (×1500) and F (×10,000), *G. prisca* from Canada (K91); E: whole colony, F: detail of the surface. In sharp contrast with the Baltic samples, cell voids (**v**) open to colony surface (similar morphology of colonies, i.e. an "open wall" type, was also observed from the GOR kerogen). Scale bar: 10 μm for Figs A, B, C, E; 1 μm for Figs D, F.



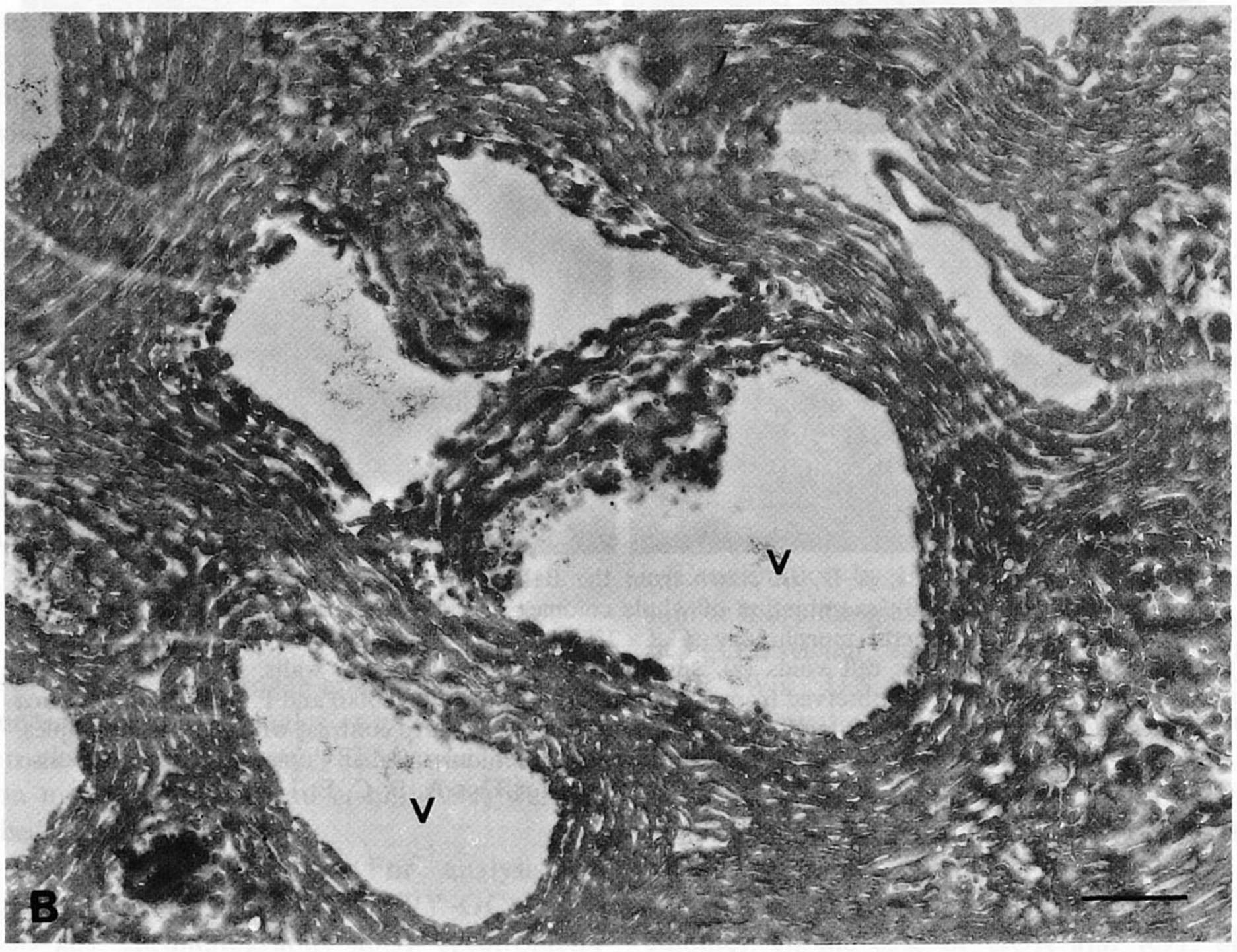


Plate 2. TEM of *G. prisca* from Estonian kukersite K1. A (×6500) and B (×10,000). The walls surrounding the cell voids (v) consist of numerous concentric and irregularly thickened layers. Scale bar: 1 μm.

distinct morphologies, which were not apparent from light microscopy observations, "closed wall" and "open wall" type, respectively.

Previous electron microscopy studies of *G. prisca*-rich kerogens were limited to two Estonian kukersites (Burns, 1982; Foster *et al.*, 1989b) and one

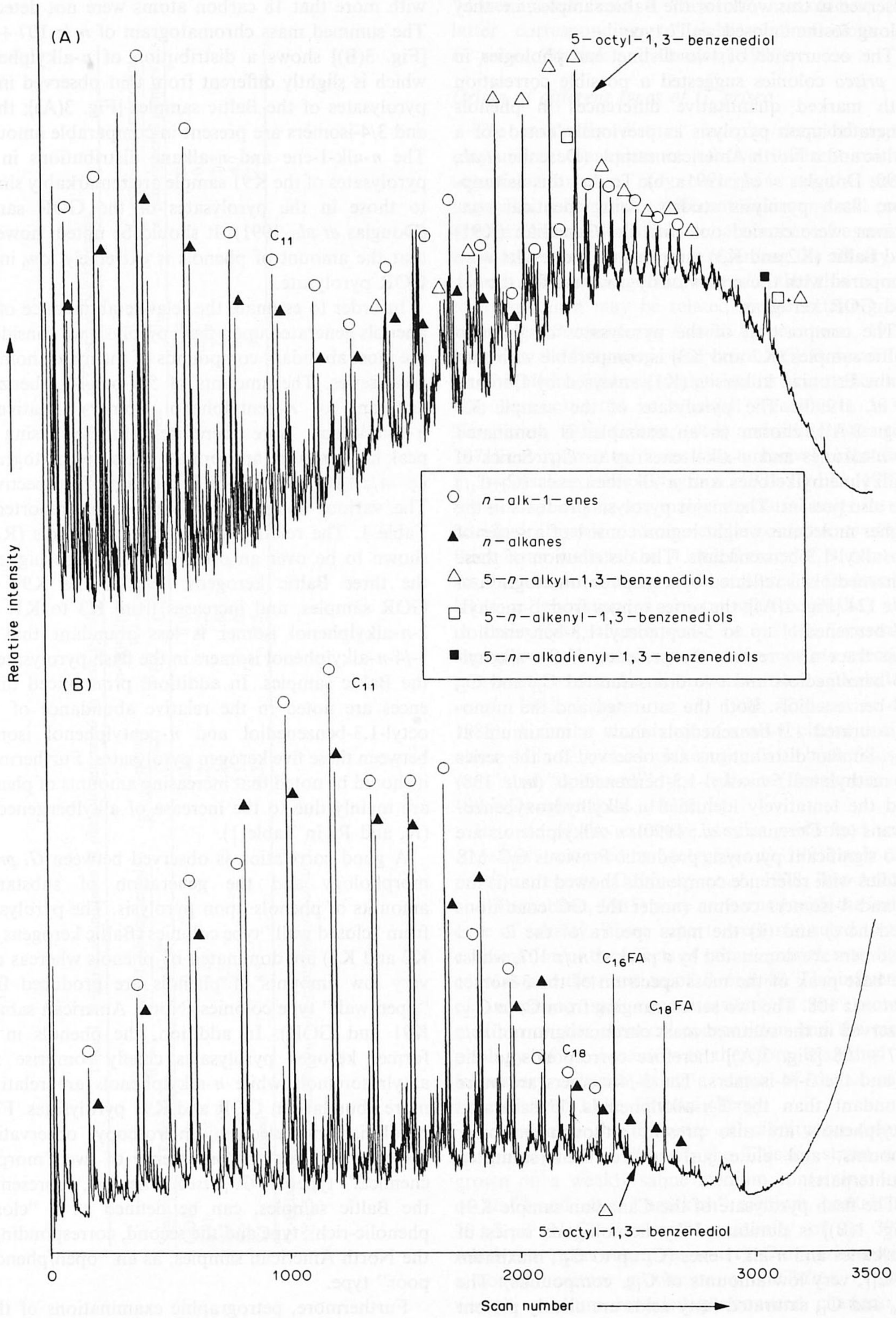


Fig. 1. Reconstructed ion chromatograms of samples K2 (A) and K91 (B) generated by flash-pyrolysis-GC–MS (Curie-temperature 610°C). C_{11} and C_{18} refer to *n*-alkane and *n*-alk-1-ene doublets with 11 and 18 carbon atoms respectively.

Ordovician kerogen from the Georgina Basin (Queensland, Australia; Playford and Wicander, 1988). The *G. prisca* colonies examined in these studies exhibited the same morphological features as observed in this work for the Baltic samples, i.e. they belong to the "closed wall" type.

The occurrence of two distinct morphologies in *G. prisca* colonies suggested a possible correlation with marked quantitative differences in phenols generated upon pyrolysis as previously noted for a Baltic and a North American sample (Derenne *et al.*, 1990; Douglas *et al.*, 1991a, b). To test this assumption, flash pyrolysis studies, using identical conditions, were carried out with the Canadian (K91) and Baltic (K2 and K3) samples and the results were compared with those previously obtained for the K1 and GOR kerogens.

The composition of the pyrolysates of the two Baltic samples (K2 and K3) is comparable with that of the Estonian kukersite (K1) analysed by Derenne *et al.* (1990). The pyrolysate of the sample K2 [Fig. 1(A)], chosen as an example, is dominated by *n*-alkanes and *n*-alk-1-enes up to C_{18}. Series of *n*-alkylmethylketones and *n*-alkylbenzenes (C_7–C_{11}) are also present. The major pyrolysis products in the higher molecular-weight region consist of a series of 5-*n*-alkyl-1,3-benzenediols. The distribution of these benzenediols is reflected by a mass chromatogram of *m/z* 124 [Fig. 2(A)]; the series ranges from 5-methyl-1,3-benzenediol up to 5-heptadecyl-1,3-benzenediol. This trace also reveals the presence of 5-*n*-alkenyl-1,3-benzenediols and two diunsaturated C_{21} and C_{23} 1,3-benzenediols. Both the saturated and the monounsaturated 1,3-benzenediols show a maximum at C_{14}. Similar distributions are observed for the series of methylated 5-*n*-alkyl-1,3-benzenediols (*m/z* 138) and the tentatively identified *n*-alkylhydroxybenzofurans (cf. Derenne *et al.*, 1990). *n*-Alkylphenols are also significant pyrolysis products. Previous GC–MS studies with reference compounds showed that (i) the 3- and 4-isomers coelute (under the GC conditions used here) and (ii) the mass spectra of the 2- and 4-isomers are dominated by a peak at *m/z* 107, whilst the base peak of the mass spectrum of the 3-isomer is at *m/z* 108. The two series, ranging from C_7 to C_{22}, observed in the summed mass chromatogram of *m/z* 107 + 108 [Fig. 3(A)] therefore corresponds to the 2- and the 3-/4-isomers. The 3-/4-isomers are more abundant than the 2-*n*-alkylphenols. Unsaturated alkylphenols are also present although in lower amounts, and elute just before their saturated counterparts.

The flash pyrolysate of the Canadian sample K91 [Fig. 1(B)] is dominated by homologous series of *n*-alkanes and *n*-alk-1-enes (C_6 up to C_{23}, maximum at C_{11}, very low amounts of C_{19+} compounds). The C_{16} and C_{18} saturated fatty acids are clearly present in the pyrolysate. The series of 5-*n*-alkyl-1,3-benzenediols is also present, but in much lower relative abundance when compared to the Baltic samples [cf. Fig. 1(A and B)]. The mass chromatogram of *m/z* 124 [Fig. 2(B)] shows that the 1,3-benzenediols have a similar distribution to those observed for the Northern Europe kerogens, although compounds with more that 18 carbon atoms were not detected. The summed mass chromatogram of *m/z* 107 + 108 [Fig. 3(B)] shows a distribution of *n*-alkylphenols which is slightly different from that observed in the pyrolysates of the Baltic samples [Fig. 3(A)]; the 2- and 3/4-isomers are present in comparable amounts. The *n*-alk-1-ene and *n*-alkane distributions in the pyrolysates of the K91 sample are remarkably similar to those in the pyrolysates of the GOR sample (Douglas *et al.*, 1991). It should be noted, however, that the amount of phenols is extremely low in the GOR pyrolysate.

In order to estimate the relative abundance of the phenols generated upon flash pyrolysis we considered the most abundant compounds of the major homologous series. The amounts of 5-*n*-octyl-1,3-benzenediol and of *n*-pentylphenol isomers, relative to *n*-octadecane, were therefore estimated using the peak heights in the appropriate mass chromatograms, i.e. *m/z* 124, *m/z* 107 + 108 and *m/z* 57, respectively. The various ratios thus obtained are reported in Table 1. The relative abundance of phenols (R_5) is shown to be over an order of magnitude higher in the three Baltic kerogens than in the K91 and GOR samples, and increases from K3 to K1. The 2-*n*-alkylphenol isomer is less abundant that the 3-/4-*n*-alkylphenol isomers in the flash pyrolysates of the Baltic samples. In addition, pronounced differences are noted in the relative abundance of 5-*n*-octyl-1,3-benzenediol and *n*-pentylphenol isomers between these five kerogen pyrolysates. Furthermore, it should be noted that increasing amounts of phenols are mainly due to the increase of alkylbenzenediols (R_5 and R_6 in Table 1).

A good correlation is observed between *G. prisca* morphology and the generation of substantial amounts of phenols upon pyrolysis. The pyrolysates from "closed wall" type colonies (Baltic kerogens K1, K2 and K3) are dominated by phenols whereas only very low amounts of phenols are produced from "open wall" type colonies (North American samples K91 and GOR). In addition, the phenols in the former kerogen pyrolysates chiefly comprise 5-*n*-alkylresorcinols while *n*-alkylphenols are relatively more abundant in GOR and K91 pyrolysates. Flash pyrolysis and electron microscopy observations therefore indicate the occurrence of two "morpho/chemical" types in *G. prisca*. The first, representing the Baltic samples, can be defined as a "closed/phenolic-rich" type and the second, corresponding to the North American samples, as an "open/phenolic-poor" type.

Furthermore, petrographic examinations of these five Ordovician deposits indicated that the "open wall" type colonies are dispersed in the mineral matrix whereas the "closed wall" type ones are

Table 1. Relative abundances of phenols generated by flash pyrolysis (Curie-temperature 610°C) of *G. prisca*-rich kerogens

Sample	Type	$R_1 = \frac{\text{5-}n\text{-octyl-1,3-benzenediol}}{C_{18}{}^*}$	$R_2 = \frac{\text{2-isomer}}{C_{18}{}^*}$	$R_3 = \frac{\text{3-/4-isomers}}{C_{18}{}^*}$	$R_4 = \frac{\text{2-isomer}}{\text{3-/4-isomers}}$	$R_5 = \frac{\Sigma\dagger}{C_{18}{}^*}$	$R_6 = \frac{\text{5-}n\text{-octyl-1,3-benzenediol}}{n\text{-pentylphenol isomers}}$
GOR	open	0	0.04	0.04	1	0.08	0
K91	open	0.2	0.2	0.2	1	0.6	0.5
K3	closed	4.7	1.1	1.6	0.7	7.4	1.7
K2	closed	6.9	0.5	1.0	0.5	8.4	4.7
K1	closed	8.6	0.2	0.5	0.4	9.3	12.2

*n-Octadecane.
†Σ = 5-n-octyl-1,3-benzenediol + n-pentylphenol isomers.

tightly packed. These features might be correlated with the occurrence of two distinct microfacies in the *G. prisca*-rich intervals of the Yeoman Formation (Ordovician, Williston basin, Canada, U.S.A.): a "disseminated" and a "layered" microfacies, the latter corresponding to tight accumulations of *G. prisca* (Stasiuk and Osadetz, 1990).

Origin of different types of G. prisca

The markedly distinct morpho/chemical types may reflect the existence of two different microorganisms or they may correspond to the same organism, living under different environmental conditions. As mentioned above, a number of assumptions have been put forward regarding the affinity of *G. prisca*. It was suggested, *inter alia*, that this marine microorganism may be related to the extant green microalga *Botryococcus braunii* (Lindenbeim, 1921) based on the following observations: (i) *B. braunii* is an ubiquitous freshwater alga but its occurrence has also been reported in brackish (Blackburn, 1936; Temperley, 1936; Gilson, 1964) and saline (Masters, 1971) environments; it was also tentatively identified in hypersaline lakes (Playford, 1977; Bauld, 1981). (ii) *n*-Alkyl chains are major structural units in the non-hydrolysable macromolecular constituents of the thick outer walls in the *A* and *B* races of *B. braunii*, (as shown from samples either collected in freshwater lakes or laboratory grown on salt-free media) and in their fossil counterparts, i.e. in the lacustrine kerogens (Torbanites) which are formed *via* selective preservation of such resistant constituents (Berkaloff *et al.*, 1983; Largeau *et al.*, 1986; Kadouri *et al.*, 1988). A similar abundance of *n*-alkyl chains is also observed in *G. prisca*-rich kerogens with both "closed wall" and "open wall" type colonies (Derenne *et al.*, 1990; Douglas *et al.*, 1991a, b).

In spite of the extensive studies performed with *B. braunii* (reviewed by Metzger *et al.*, 1991) only two previous reports (Dubinsky *et al.*, 1978; Ben-Amotz and Tornabene, 1985) were concerned with the influence of salinity. These reports were restricted to the examination of the effects of salt on growth rate, lipid content and distribution of *B. braunii*. The influence of increasing salt concentrations in the growth medium on the chemical composition and the morphology of *B. braunii* was therefore examined in laboratory cultures using a strain of the *A* race isolated from Lake Titicaca (Peru).

To examine the influence of salinity on colony morphology of *B. braunii*, the Titicaca strain was grown on a weakly saline medium containing 1 g/l of NaCl and on a "brackish" medium, containing 10 g/l of NaCl (i.e. with a salinity of *ca* 30% relative to open ocean sea water). Comparison with a control culture grown on a NaCl-free, "freshwater", medium revealed pronounced changes in morphology (Plate 3). The control culture shows the classical morphological features of *B. braunii* with only the basal part of cells embedded in a

rather thick layered outer wall. This general organization strongly resembled the one observed in the present study for the "open wall" type colonies of *G. prisca*. The culture grown on a low salinity (1 g/l) medium is characterized by a marked increase in thickness and lamination of basal walls. Inward folding of the tip of the basal walls also occurs; as a result, the apical part of some cells is now

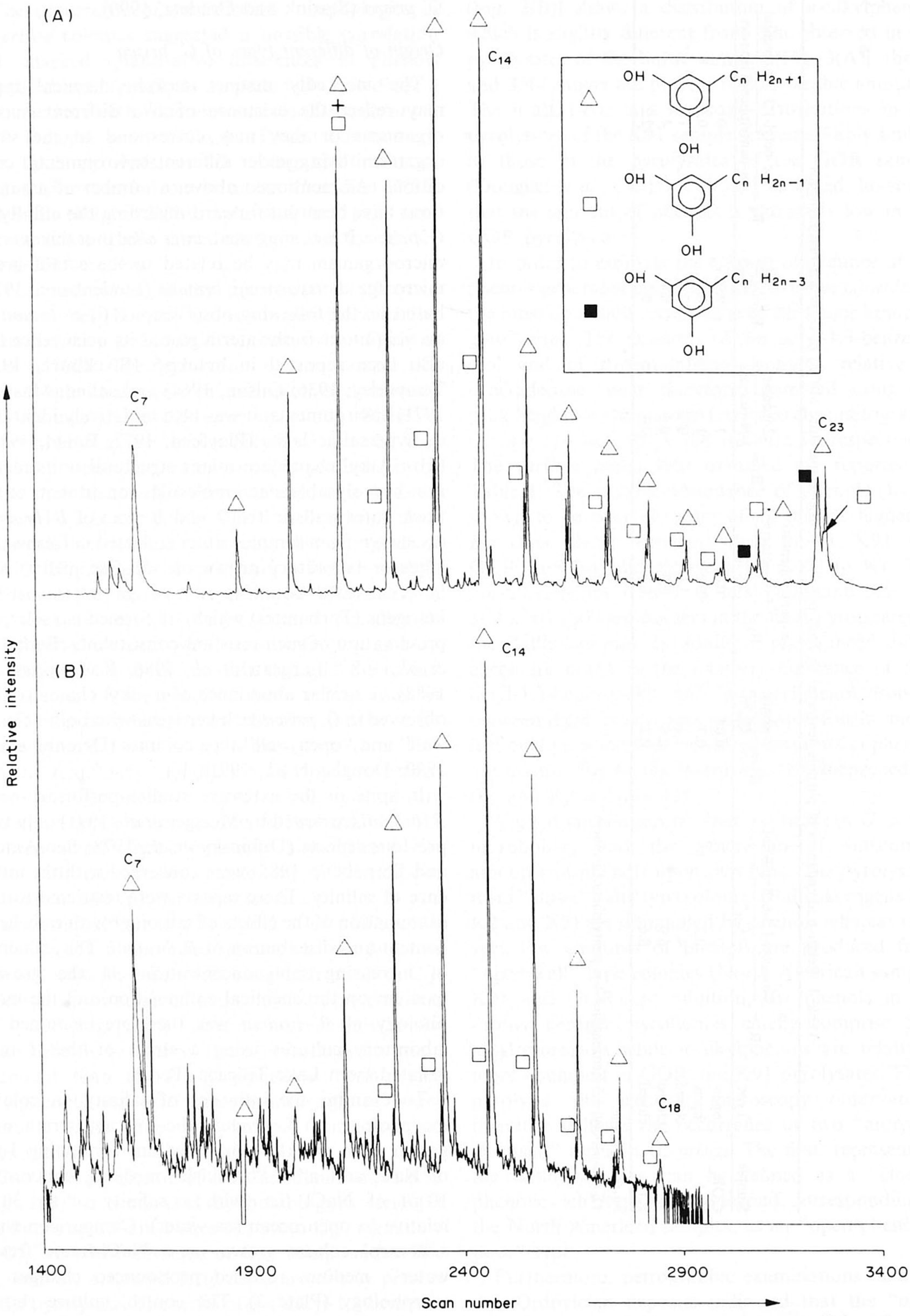


Fig. 2. Mass chromatograms *m/z* 124 showing the distribution patterns of 5-*n*-alkyl-1,3-benzenediols and their mono- and diunsaturated counterparts in the flash pyrolysate of samples K2 (A) and K91 (B).

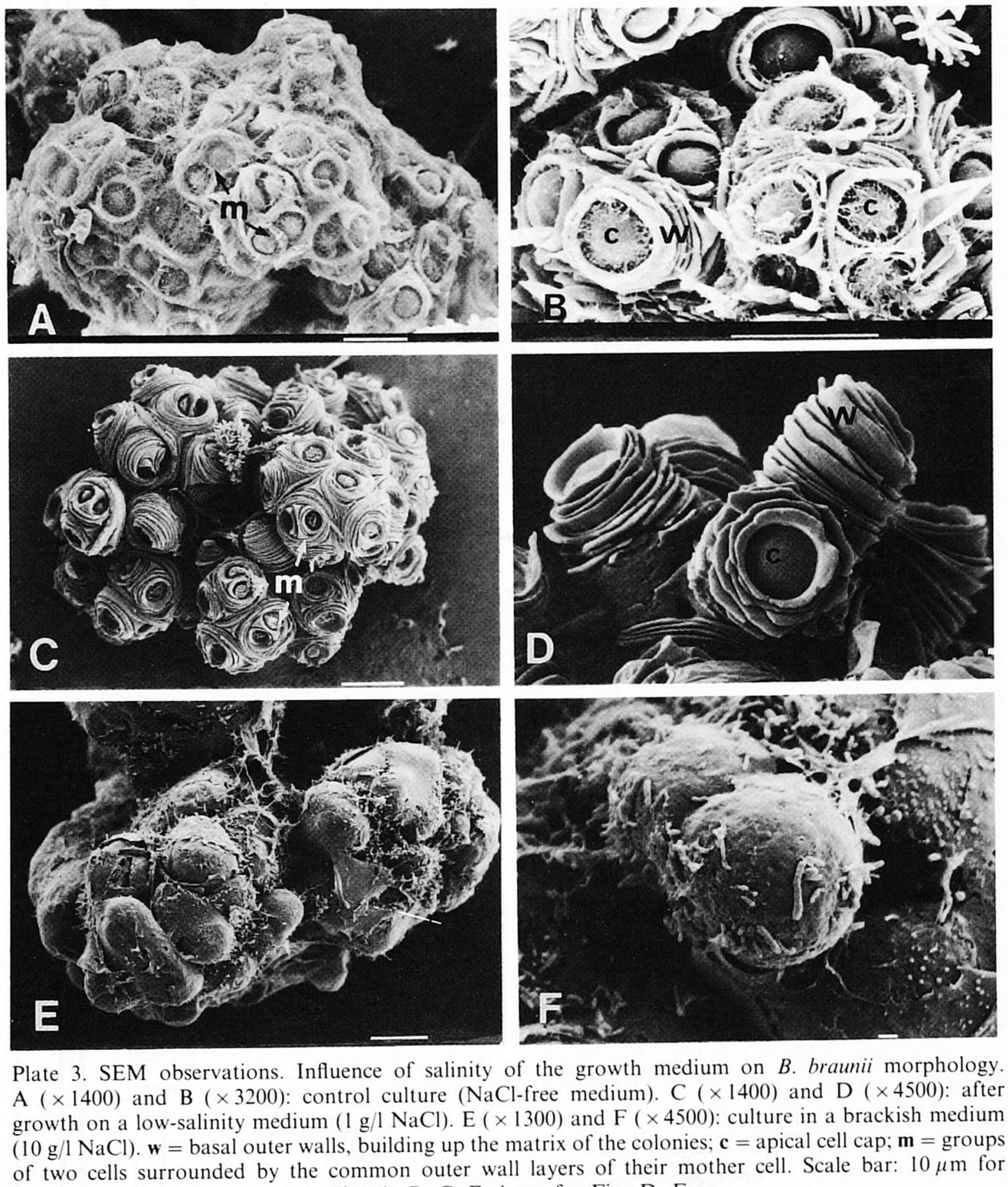


Plate 3. SEM observations. Influence of salinity of the growth medium on *B. braunii* morphology. A (×1400) and B (×3200): control culture (NaCl-free medium). C (×1400) and D (×4500): after growth on a low-salinity medium (1 g/l NaCl). E (×1300) and F (×4500): culture in a brackish medium (10 g/l NaCl). **w** = basal outer walls, building up the matrix of the colonies; **c** = apical cell cap; **m** = groups of two cells surrounded by the common outer wall layers of their mother cell. Scale bar: 10 μm for Figs A, B, C, E; 1 μm for Figs D, F.

partly hidden by these multilamellate walls. Colonies from the "brackish" cultures show a markedly different organization. The basal and apical parts of cells are no more distinct and a close resemblance is noted with the "closed wall" type colonies of *G. prisca*.

The pronounced morphological changes in *B. braunii* colonies observed suggest that the "open

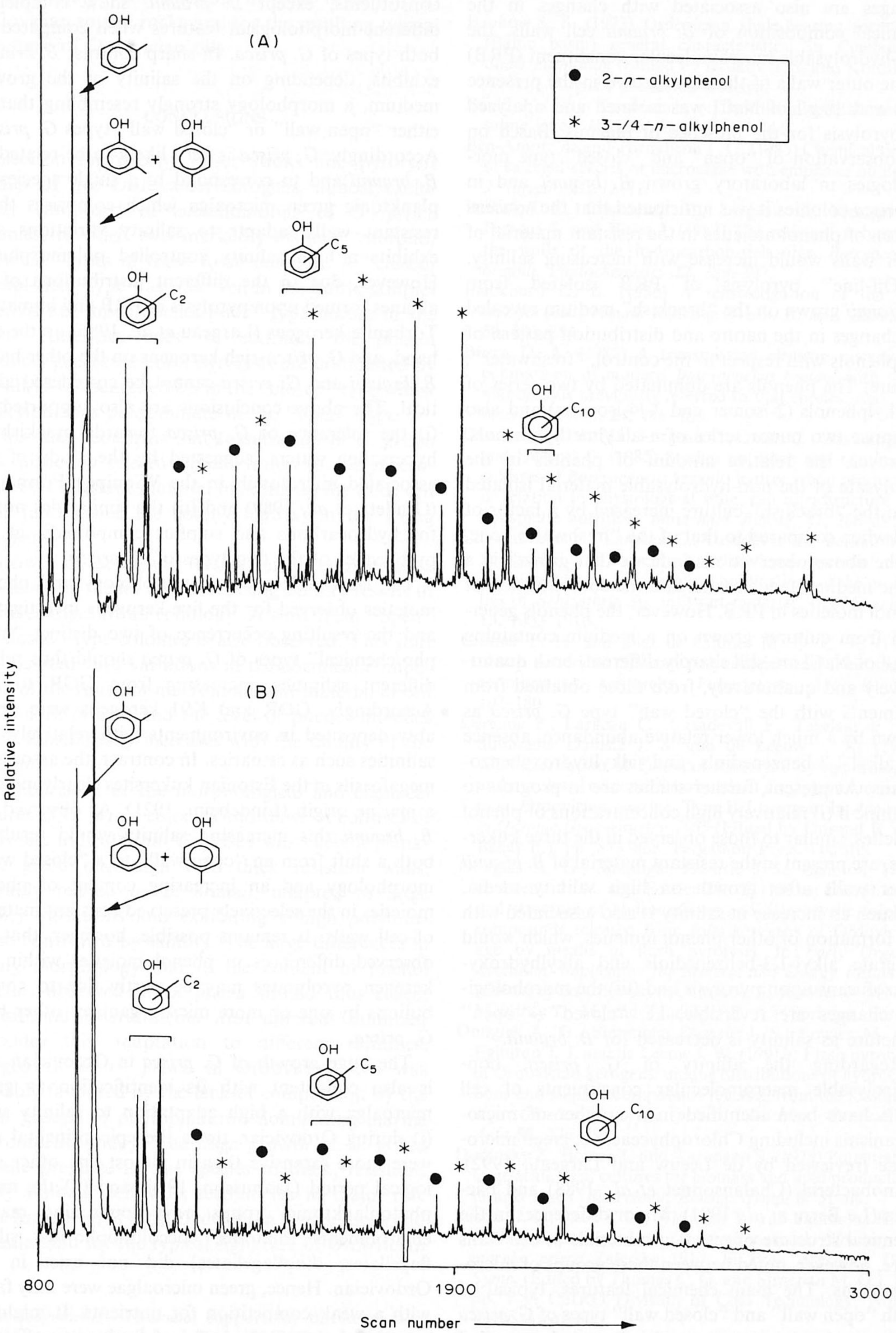


Fig. 3. Summed mass chromatograms m/z 107 + 108 showing the distribution patterns of *n*-alkylphenols in the flash pyrolysates of samples K2 (A) and K91 (B).

wall" and "closed wall" types of *G. prisca* may correspond to a colonial alga, possibly related to *B. braunii*, living in environments with different salinities.

To verify whether the above morphological changes are also associated with changes in the chemical composition of *B. braunii* cell walls, the non-hydrolysable macromolecular constituent (PRB) of the outer walls of the algae grown in the presence of 0 and 10 g/l of NaCl was isolated and analyzed by pyrolysis for the presence of phenols. Based on the observation of "open" and "closed" type morphologies in laboratory grown *B. braunii* and in *G. prisca* colonies it was anticipated that the concentration of phenol moieties in the resistant material of outer walls would increase with increasing salinity.

"Off-line" pyrolysis of PRB isolated from *B. braunii* grown on the "brackish" medium revealed no changes in the nature and distribution pattern of the phenols with respect to the control, "freshwater", culture. The phenols are dominated by two series of *n*-alkylphenols (2-isomer and 3-/4-isomers) and also comprise two minor series of *n*-alkylmethylphenols. However, the relative amount of phenols in the pyrolysate of the non-hydrolysable material isolated from the "brackish" culture increased by a factor of *ca* 4 when compared to that of the "freshwater" one.

The above observations indicate that growth in a saline medium tends to increase the concentration of phenol moieties in PRB. However, the phenols generated from cultures grown on a medium containing 10 g/l of NaCl are still sharply different, both quantitatively and qualitatively, from those obtained from sediments with the "closed wall" type *G. prisca* as shown by a much lower relative abundance, absence of alkyl-1,3-benzenediols and alkylhydroxybenzofurans. At present, further studies are in progress to examine if (i) relatively high concentrations of phenol moieties, similar to those observed in the three kukersites, are present in the resistant material of *B. braunii* outer walls after growth on high salinity media, (ii) such an increase in salinity is also associated with the formation of other phenol moieties, which would generate alkyl-1,3-benzenediols and alkylhydroxybenzofurans upon pyrolysis and (iii) the morphological changes are reversible, i.e. "closed" → "open" structure as salinity is decreased for *B. braunii*.

Regarding the affinity of *G. prisca*, non-hydrolysable macromolecular constituents of cell walls have been identified in a number of microorganisms including Chlorophyceae, i.e. green microalgae (reviewed by de Leeuw and Largeau, 1992), cyanobacteria (Chalansonnet *et al.*, 1988) and bacteria (Le Berre *et al.*, 1991). Major differences in the chemical structure of resistant materials of cell walls were, however, noted between these groups of microorganisms. The main chemical features, typical of both "open wall" and "closed wall" types of *G. prisca* (macromolecular structure dominated by *n*-alkyl chains, presence of substantial amounts of *n*-alkylcyclohexyl groups and very low contribution of acyclic isoprenoids in pyrolysates) have been noted only in the resistant outer walls of green microalgae. However, all the Chlorophyceae so far shown to comprise non-hydrolysable macromolecular wall constituents, except *B. braunii*, show completely different morphological features when compared to both types of *G. prisca*. In sharp contrast *B. braunii* exhibits, depending on the salinity of the growth medium, a morphology strongly resembling that of either "open wall" or "closed wall" types *G. prisca*. Accordingly, *G. prisca* is very likely to be related to *B. braunii* and to correspond to a single species of planktonic green microalga which comprises thick resistant walls, adapts to salinity variations and exhibits a high salinity controlled polymorphism. However, due to the different distributions of *n*-alkanes formed upon pyrolysis of PRB and immature Torbanite kerogens (Largeau *et al.*, 1986) on the one hand, and *G. prisca*-rich kerogens on the other hand, *B. braunii* and *G. prisca* cannot be considered identical. The above conclusions are also supported by (i) the tolerance of *G. prisca* towards brackish to hypersaline waters, suggested by the study of the associated microfossils in the Winnipeg Formation (Osadetz *et al.*, 1989) and (ii) the similarities noted, for hydrocarbons and sulphur compounds, in the pyrolysates of the two types of *G. prisca*.

The large differences in morphology and phenol moieties observed for the five kerogens investigated, and the resulting occurrence of two distinct "morpho/chemical" types of *G. prisca* should thus reflect different salinities, increasing from GOR to K1. Accordingly, GOR and K91 kerogens were probably deposited in environments with relatively low salinities such as estuaries. In contrast, the associated megafossils in the Estonian kukersites clearly indicate a marine origin (Lindebeim, 1921). As observed for *B. braunii* this increasing salinity would result in both a shift from an "open wall" to a "closed wall" morphology and an increasing content of phenol moieties in the selectively preserved resistant material of cell walls. It remains possible, however, that the observed differences in phenol moieties within the kerogen pyrolysates may be partly due to contributions by one or more microorganisms other than *G. prisca*.

The huge growth of *G. prisca* in Ordovician seas is also consistent with its identification as green microalga with a high adaptation to salinity since (i) during Ordovician times the epicontinental seas were more extensive than in almost any other geological period (Jaanusson, 1984) and (ii) the major phytoplanktonic groups now dominating marine environments (diatoms, coccolithophorids, silicoflagellates, dinoflagellates) did not exist in the Ordovician. Hence, green microalgae were only faced with a weak competition for nutrients. It might be speculated that *B. braunii* is a freshwater offspring of *G. prisca* species which apparently could easily

adapt to changes in salinity. The abundant growth of *G. prisca*, in combination with the occurrence of thick, resistant and highly aliphatic outer walls exhibiting a specific composition and having been selectively preserved during diagenesis, accounts for the major contribution of this organism to Ordovician source rocks and for the resulting typical signature of Ordovician oils.

CONCLUSIONS

Electron microscopy observations and pyrolysis studies of five Ordovician kerogens, almost exclusively composed of accumulations of *G. prisca* colonies, revealed two markedly distinct "morpho/chemical" types in these fossil colonies. A "closed/phenol-rich" type was observed in the Baltic samples whereas an "open/phenol-poor" type occurs in the North American ones. In addition, the phenol products generated upon pyrolysis are dominated by 5-*n*-alkyl-1,3-benzenediols in the "closed" type whilst *n*-alkylphenols predominate in the "open" type.

This study confirms that fossilisation of *G. prisca* took place *via* selective preservation of the macromolecular resistant material building up the thick cell walls (and hence the colony matrix) in the living organism.

Growth of the extant, freshwater, green microalga *B. braunii* on media with increasing salinity results in major changes in morphology. A shift from "open" to "closed" type colonies is also observed. This shift is associated with variations in the chemical composition of the resistant macromolecular material of cell walls of *B. braunii* and the level of phenol moieties which substantially increases with the salinity of the medium.

Comparisons of the morphological and chemical features of *G. prisca* colonies and those of cultures of *B. braunii* indicate that *G. prisca* was likely a planktonic green microalga with thick resistant walls, probably related to *B. braunii*, adapted to large salinity variations and exhibiting a high polymorphism controlled by salinity. The large differences in colony morphology and in the content of phenol moieties observed in *G. prisca* should thus reflect depositional environments with different salinities.

Besides this adaptation to different salinities, the growth of *G. prisca* in Ordovician seas was probably favoured by the lack of competition by the major groups of phytoplankton dominating marine environments today. The huge growth of *G. prisca* and the presence in this species of thick, resistant, highly aliphatic cell walls account for its major role in the formation of most Ordovician source rocks and oil shales and for the typical signature of Ordovician oils.

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REFERENCES

Baukov S. S. (1973) Ordovician shale-bearing formation (Baltic basin) general characteristics. In *Combustible Shale Formation* (Edited by Baukov S. S. and Kotlukova V. A.). Valgus Publishers, Moscow.

Bauld J. (1981) Occurrence of benthic microbial mats in saline lakes. *Hydrobiologia* **81,** 87–111.

Ben-Amotz A. and Tornabene T. G. (1985) Chemical profile of selected species of microalgae with emphasis on lipids. *J. Phycol.* **21,** 72–81.

Berkaloff C., Casadevall E., Largeau C., Metzger P., Peracca S. and Virlet J. (1983) The resistant polymer of the walls of the hydrocarbon-rich alga *Botryococcus braunii*. *Phytochemistry* **22,** 389–397.

Blackburn K. B. (1936) A reinvestigation of the alga *Botryococcus braunii* Kützing. *Trans. R. Soc. Edinburgh* **58,** 841–854.

Burns D. A. (1982) A transmission electron microscope comparison of modern *Botryococcus braunii* with some microfossils previously referred to that species. *Rev. Esp. Micropaleontol.* **24,** 165–185.

Casadevall E., Dif D., Largeau C., Gudin C., Chaumont D. and Desanti O. (1985) Studies on batch and continuous cultures of *Botryococcus braunii*: hydrocarbon production in relation to physiological state, cell ultrastructure and phosphate nutrition. *Biotechnol. Bioeng.* **27,** 286–295.

Chalansonnet S., Largeau C., Casadevall E., Berkaloff C., Péniguel G. and Couderc R. (1988) Cyanobacterial resistant biopolymers. Geochemical implications of the properties of *Schizothrix* sp. resistant material. *Org. Geochem.* **13,** 1003–1010.

Cramer F. H. and Diez de Cramer M. D. C. R. (1972) North American Silurian palynofacies and their spatial arrangement: acritarchs. *Palaeontogr. Abt. B.* **138,** 107–180.

Derenne S., Largeau C., Casadevall E., Tegelaar E. W., Sinninghe Damsté J. S. and de Leeuw J. W. (1990) Characterisation of Estonian Kukersite by spectroscopy and pyrolysis: evidence for abundant alkylphenol moieties in an Ordovician, marine, Type II/I kerogen. In *Advances in Organic Geochemistry 1989* (Edited by Durand B. and Behar F.), pp. 873–888. Pergamon Press, Oxford.

Douglas A. G., Sinninghe Damsté J S., Eglinton T. I., de Leeuw J. W. and Fowler M. G. (1991a) Distribution and structure of hydrocarbons and heterocyclic sulphur compounds released from four kerogens of Ordovician age by means of flash pyrolysis. In *Early Organic Evolution: Implication for Mineral and Energy Resources* (Edited by Schidlowski M., Golubic S., Kimberley M. M., McKirdy D. M. and Trudinger P. A.). Springer, Berlin.

Douglas A. G., Sinninghe Damsté J. S., Fowler M. G., Eglinton T. I. and de Leeuw J. W. (1991b) Flash pyrolysis of Ordovician kerogens: unique distributions of hydrocarbons and sulphur compounds released from the fossilized alga *Gloeocapsomorpha prisca*. *Geochim. Cosmochim. Acta* **55,** 275–291.

Dubinsky Z., Berner T. and Aaronson S. (1978) Potential of large-scale algal cultures for biomass and lipid production in arid lands. *Biotechnol. Bioeng. Symp.* **8,** 51–68.

Foster C. B., Reed J. D., Wicander R. and Summons R. E. (1989a) Anatomy of an oil-prone maceral: *Gloeocapsomorpha prisca* Zalessky 1917. In *Proc. Macerals 1989 Symp.* (Edited by Thomas C. G. and Strachan M. G.), **16,** pp. 1–9. CSIRO Division of Coal Technology, North Ryde, Australia.

Foster C. B., Reed J. D. and Wicander R. (1989b) *Gloeocapsomorpha prisca* Zalessky 1917: a new study.

Part I: Taxonomy, Geochemistry and Paleoecology. *Geobios*. **22,** 735–759.

Foster C. B., Wicander R. and Reed J. D. (1990) *Gloeocapsomorpha prisca* Zalessky, 1917: a new study. Part II: Origin of Kukersite, a new interpretation. *Geobios*. **23,** 133–140.

Fowler M. G. (1984) Organic geochemistry of pre-Carboniferous sedimentary organic matter. Ph.D. thesis, Univ. of Newcastle-upon-Tyne.

Fowler M. G. (1991) The influence of *Gloeocapsomorpha prisca* on the organic geochemistry of oils and organic-rich rocks of late Ordovician age from Canada. In *Early Organic Evolution: Implications for Mineral and Energy Resources* (Edited by Schidlowski M., Golubic S., Kimberley M. M., McKirdy D. M. and Trudinger P. A.). Springer, Berlin.

Fowler M. G. and Douglas A. G. (1984) Distribution and structure of hydrocarbons in four organic-rich Ordovician rocks. In *Advances in Organic Geochemistry 1983* (Edited by Schenck P. A., de Leeuw J. W. and Lijmbach G. W. M.), pp. 105–114. Pergamon Press, Oxford.

Fowler M. G., Abolins P. and Douglas A. G. (1986) Monocyclic alkanes in Ordovician organic matters. In *Advances in Organic Geochemistry 1985* (Edited by Leythaeuser D. and Rullkötter J.), pp. 815–823. Pergamon Press, Oxford.

Gilson H. C. (1964) "Lake Titicaca". *Int. Ass. Theor. Appl. Limnol.* **15,** 112–127.

Glikson M., Lindsay K. and Saxby J. (1989) *Botryococcus*—A planktonic green alga, the source of petroleum through the ages: transmission electron microscopical studies of oil shales and petroleum source rocks. *Org. Geochem.* **14,** 595–608.

Hatch J. R., Jacobson S. R., Witzke B. J., Risatti J. B., Anders D. E., Watney W. L., Newell K. D. and Vuletich A. K. (1987) Possible Late Middle Ordovician organic carbon isotope excursions: evidence from Ordovician oils and hydrocarbon source rocks, mid-continent and east-central United States. *Am. Assoc. Pet. Geol. Bull.* **71,** 1342–1354.

Hoffmann C. F., Foster C. B., Powell T. G. and Summons R. E. (1987) Hydrocarbon biomarkers from Ordovician sediments and the fossil alga *Gloeocapsomorpha prisca* Zalessky 1917. *Geochim. Cosmochim. Acta* **51,** 2681–2697.

Jaanusson V. (1984) What is so special about the Ordovician. In *Aspects of the Ordovician system* (Edited by Bruton D. L.) Paleontological contributions from the University of Oslo No. 295, Universitetsforlaget, 1–3.

Jacobson S. R., Hatch J. R., Teerrman S. C. and Askius R. A. (1988) Middle Ordovician organic matter assemblages and their effect on Ordovician-derived oils. *Bull. Am. Assoc. Pet. Geol.* **58,** 499–506.

Kadouri A., Derenne S., Largeau C., Casadevall E. and Berkaloff C. (1988) Resistant biopolymer in the outer walls of *Botryococcus braunii*, *B* race. *Phytochemistry* **27,** 551–557.

Klesment I. (1974) Application of chromatographic methods in biogeochemical investigations. Determination of the structures of sapropelites by thermal decomposition. *J. Chromatogr.* **91,** 705–713.

Klesment I. and Nappa L. (1980) Investigation of the structure of Estonian oil shale Kukersite by conversion in aqueous suspension. *Fuel* **59,** 117–122.

Konzalova M. (1973) Algal colony and rests of other microorganisms in the Bohemian Upper Proterozoic. *Vestmik Ustredniho Ustavu Geol.* **48,** 31–34.

Largeau C., Derenne S., Casadevall E., Kadouri A. and Sellier N. (1986) Pyrolysis of immature Torbanite and of the resistant biopolymer (PRB *A*) isolated from extant alga *Botryococcus braunii*. Mechanism of formation and structure of Torbanite. *Org. Geochem.* **10,** 1023–1032.

Largeau C., Derenne S., Clairay C., Casadevall E., Raynaud J. F., Lugardon B., Berkaloff C., Corolleur M. and Rousseau B. (1990) Characterization of various kerogens by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Morphological relationship with resistant outer walls in extant micro-organisms. *Meded. Rijks. Geol. Dienst.* **45,** 91–101.

Le Berre F., Derenne S., Largeau C., Connan J. and Berkaloff C. (1991) Occurrence of non-hydrolysable, macromolecular, wall constituents in bacteria. Geochemical implications. In *Organic Geochemistry. Advances and Applications in the Natural Environment* (Edited by Manning D. A. C.), pp. 428–431. Manchester University Press, Manchester.

de Leeuw J. W. and Largeau C. (1992) A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal and petroleum formation. In *Organic Geochemistry* (Edited by Engel M. H. and Macko S. A.). Plenum Publishing Corp., New York.

Lindenbeim H. A. K. (1921) La Kukersite, Etude d'un dépôt marin phytogène du silurien inférieur d'Estonie. *Arch. Sci. Phys. Nat.* **3,** 379–413.

Longman M. W. and Palmer S. E. (1987) Organic geochemistry of Mid-Continent Middle and Late Ordovician oils. *Am. Assoc. Pet. Geol. Bull.* **71,** 938–950.

Martin R. L., Winters H. C. and Williams J. A. (1963) Distribution of *n*-paraffins in crude oils and their implications to the origin of petroleum. *Nature, London* **199,** 110–113.

Masters M. J. (1971) The occurrence of *Chrytridium marylandicum* on *Botryococcus braunii* in School Bay of the Delta Marsh. *Can. J. Bot.* **49,** 1479–1485.

Metzger P., Largeau C. and Casadevall E. (1991) Lipids and macromolecular lipids of the hydrocarbon-rich microalga *Botryococcus braunii*. Chemical structure and biosynthesis—Geochemical and Biotechnological importance. In *Progress in the Chemistry of Organic Natural Products, 57* (Edited by Herz W., Grisebach H., Kirby G. W. and Tamun C.), pp. 1–70. Springer, Wein.

Monin J. C., Durand B., Vandenbroucke M. and Huc A. Y. (1980) Experimental simulation of the natural transformation of kerogen. In *Advances in Organic Geochemistry 1979* (Edited by Douglas A. G. and Maxwell J. R.), pp. 517–529. Pergamon Press, Oxford.

Osadetz K. G., Snowdon L. R. and Stasiuk L. D. (1989) Association of enhanced hydrocarbon generation and crustal structure in the Canadian Williston Basin. *Curr. Res. Part D*. 35–47.

Playford P. E. (1977) Geology and ground water potential. In *Geology and Hydrology of Rottnest Islands* (Edited by Playford P. E. and Leach R. E. J.), pp. 1–53. Geol. Surv. W. A. Rep. 6.

Playford G. and Wicander R. (1988) Acritarch palynoflora of the Coolibah Formation (Lower Ordovician), Georgina Basin, Queensland. *Mem. Assoc. Australas. Palaeontols.* **5,** 5–40.

Powell T. G. and McKirdy D. M. (1972) The geochemical characterization of Australian crude oils. *Aust. Pet. Explor. Assoc. J.* **55,** 125–131.

Reed J. D., Illich H. A. and Horsfield B. (1986). Biochemical evolutionary significance of Ordovician oils and their sources. In *Advances in Organic Geochemistry 1985* (Edited by Leythaeuser D. and Rullkötter J.), pp. 347–358. Pergamon Press, Oxford.

Stasiuk L. D. and Osadetz K. G. (1990) The life cycle and phyletic affinity of *Gloeocapsomorpha prisca* Zalessky 1917 from Ordovician rocks in the Canadian Williston Basin. *Curr. Res. Part. D*. 123–137.

Temperley B. N. (1936) The Boghead controversy and the morphology of the Boghead algae. *Trans. R. Soc. Edinburgh* **43,** 855–868.

Traverse A. (1955) Occurrence of the oil-forming alga *Botryococcus* in lignites and other tertiary sediments. *Micropaleontology* **1,** 343–350.

Tynni R. (1975) Ordovician hystrichospheres and chitinozoans in limestone from the Bothnian sea. *Geol. Sur. Finland Bull.* **279,** 5–49.

Wielens J. B. W., Dick von der H., Fowler M. G., Brooks P. W. and Monnier F. (1990) Geochemical comparison of a Cambrian alginite potential source rock and hydrocarbons from the Colville/Tweed lake area. *Bull. Can. Petrol. Geol.* **38,** 236–245.

Zalessky M. D. (1917) On marine sapropelite of Silurian age formed by a blue-green alga. *Izv. Imp. Akad. Nauk. IV Ser.* 3–18.