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Epilithic and chasmoendolithic phycoflora of monuments and buildings in South-Eastern Spain

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Abstract – Algae and cyanobacteria occurring on monuments and other epilithic habitats in the Region of Murcia (SE Spain) were studied. Samples were collected from 16 subaerial sites, including monuments (12), calcareous rocks (3) and a new building (1); limestone and marble were the predominant substrata. Overall, 47 taxa were identified (22 cyanophytes, 4 heterokontophytes and 20 chlorophytes). In total, 58 unialgal strains were isolated. The following species were recorded for the first time for Spain: *Cyanobacterium cedrorum* (Sauv.) Komárek *et* Anagn., *Nautococcus terrestris* P.A.Archibald, *Tetracystis sarcinalis* Schwarz, *Ecdysichlamys obliqua* G.S.West, *Oocystis asymmetrica* W. *et* G.S.West sensu Komáromy, *Chlorella kessleri* Fott *et* Nováková, *Klebsormidium nitens* (Menegh. in Kütz.) Lokhorst, *Klebsormidium crenulatum* (Kütz.) Lokhorst and *Stichococcus allas* Reisigl. The most diverse genus was *Leptolyngbya* (7 taxa) and the most frequent taxa were *Chroococcidiopsis kashaii*, *Nostoc sphaericum*, *Botrydiopsis* sp., *Apatococcus lobatus*, *Chlorosarcinopsis* cf. *arenicola*, *Muriella terrestris, Chlorella vulgaris, Chlorella kessleri* and *Stichococcus bacillaris.* The epi- and (chasmo)endolithic flora from these sites showed a dominance of prokaryotic microorganisms and only the more humid sites were colonized by eukaryotic algae. The chasmoendolithic cyanophyte *Chroococcidiopsis kashaii* was dominant. The data showed that coccoid species outnumbered the filamentous species and represented 55 % of all taxa recorded. The type of lithic substratum is considered to have little or no influence on the composition of the lithic phycoflora in the localities investigated.

biodeterioration / monuments / cyanobacteria / cyanophytes / algae / biodeteriogen / Murcia / SE Spain

Résumé – **Flore des algues épilithes et chasmoendolithes des monuments et bâtiments du sud-est de l'Espagne.** Seize monuments historiques et habitats épilithes de la région de Murcie (SE l'Espagne) ont été prospectés: douze monuments, trois habitats calcaires et une nouvelle construction. Le calcaire et le marbre sont les supports prédominants. Quarante-sept taxons ont été identifiés (vingt deux Cyanophyta, quatre Heterokontophyta et vingt Chlorophyta). Tous les taxons sont décrits et dessinés. Cinquante huit cultures uni-algales ont été isolées. Neuf espèces sont nouvelles pour la flore algologique de l'Espagne: *Cyanobacterium cedrorum* (Sauv.) Komárek *et* Anagn., *Nautococcus terrestris* P.A.Archibald, *Tetracystis sarcinalis* Schwarz, *Ecdysichlamys obliqua* G.S.West, *Oocystis asymmetrica* W. *et*

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G.S.West sensu Komáromy, *Chlorella kessleri* Fott *et* Nováková, *Klebsormidium nitens* (Menegh. in Kütz.) Lokhorst, *Klebsormidium crenulatum* (Kütz.) Lokhorst *et Stichococcus allas* Reisgl. Le genre le plus diversifié est *Leptolyngbya* (7 taxons) et les espèces plus fréquentes sont *Chroococcidiopsis kashaii*, *Nostoc sphaericum*, *Botrydiopsis* sp., *Apatococcus lobatus*, *Chlorosarcinopsis* cf. *arenicola*, *Muriella terrestris, Chlorella vulgaris, Chlorella kessleri* et *Stichococcus bacillaris.* La flore épi- et chasmoendolithes des sites études présentent une dominance des microorganismes procaryotes, tandis que seules les zones les plus humides sont colonisées par des algues eucaryotes. Les formes coccoïdes sont plus nombreuses que les formes filamenteuses et représentent 55 % de toutes les formes étudiées. Nos résultats confirment la faible influence du substrat lithique sur la composition floristique des communautés algales dans les localités étudiées.

biodétérioration / monuments / cyanobactéries / cyanophycées / algues / biodeteriogen / Murcie / sud-est de l'Espagne

INTRODUCTION

The region of Murcia (SE Spain) has an important cultural heritage, including caves with prehistoric paints, Roman and Middle Age buildings and Baroque cathedrals. In this artificial environment, air-borne cells and spores settle and develop on building surfaces and form microorganism biofilms of varying thickness. Knowledge of the diversity of these lithobiont assemblages in these habitats is necessary to understand the adaptation of microorganisms to such extreme environments, and is important in predicting the potential damage due to changes of the characteristics of the substratum caused by lithophytic microrganisms.

During the last five decades, several studies, mainly conducted in Southwestern and Western Europe and using various methodological approaches, have significantly contributed to our knowledge and understanding of the biology of subaerial cyanobacteria and algae. Only a few studies of subaerial algae are available for urban habitats. Most papers on cyanobacteria and algae in urban environments have focused on biodeterioration problems. Very few contributions on subaerial algae in urban environments have examined in detail the morphology and life history of individual taxa and assemblages. Observations made by Kapusta & Kovacik (2000), Rindi & Guiry (2003) and Uher *et al.* (2003) indicate that many urban habitats host a rich and diversified subaerial algal flora. In this study, we examine the taxonomic status and autecological traits of subaerial algae and cyanobacteria occurring in urban habitats in the region of Murcia and we document in detail their distributional patterns.

MATERIALS & METHODS

The accumulation of calcium ions (Ca^{2+}) is the most important factor of the edaphogenesis in the region of Murcia. In the region, areas situated near the Mediterranean shore are characterized by a hot and arid climate. The average annual rainfall is lower than 330 mm per year and the annual temperature mainly oscillates between 6 and 14 °C in the north of the region, in which most of the

Table 1. Characteristics of sampling sites of Murcia

No.	*Name of building*	*Average annual T°C*	*City*	*Type of substratum*	*Microenvironment*	*Number of samples*	*Date*
1.	Real Monasterio de Encarnacion de Monjas Clarisas	6-14ºC	Mula	Limestone	Entrance, wall, Shadow, semiarid	3	May 1996
2.	Castillo de los Vélez	6-14ºC	Mula	Limestone	Wall, lighted, semiarid	2	May 1996
3.	Monasterio de San Francisco	6-14ºC	Mula	Brick	Wall, lighted, semiarid	4	May 1996
4.	Iglesia del Santo Cristo	6-14ºC	Cehegin	Marble	Wall, shadow, semiarid	3	May 1996
5.	Iglesia del Salvador	6-14ºC	Caravaca	Limestone	Stone, lighted. semiarid	3	May 1996
6.	Iglesia Nuestra Señora de la Asuncini	6-14ºC	Moratalla	Limestone	Stone, lighted, semiarid	4	May 1996
7.	Colegiata de San Patricio	12-15ºC	Lorca	Limestone	Stone, lighted. semiarid	2	May 1996
8.	Palacio de Guevara	12-15ºC	Lorca	Sandstone & Brick	Wall, lighted, semiarid	1	May 1996
9.	Palacio Episcopal	16-18ºC	Murcia	Limestone	Wall, shadow, humid	3	June 1996
10.	Catedral de Murcia	16-18ºC	Murcia	Marble	Wall, shadow, semiarid	5	June 1996
11.	Medina Siyasa	16-18ºC	Cieza	Limestone	Stone, lighted, semiarid	2	May 1996
12.	Castillo de Monteagudo	16-18ºC	Monteagudo	Sandstone	Wall, shadow, humid	4	May 2003
13.	Faculdad de Biologia	16-18ºC	Murcia	Concrete	Wall, shadow, humid	7	March. 2003
14.	Ulal, chalk spring	16-18ºC	Marjal de Oliva	Limestone	Rock, lighted, humid	3	April 2003
15.	Fonte Salada, chalk spring	16-18ºC	Oliva	Limestone	Cave, humid	2	May 2003
16.	La Puerta (valley)	16-18ºC	Rio Alharabe	Limestone	Rock, shadow, humid	5	May 2003

annual rainfall occurs in winter. The temperature mostly ranges between 12 and 15 ºC in the continental zone of the north of the region, characterized by high rainfall in spring. Average annual temperatures between 16 and 18 ºC characterize the south of the Region, which is the hottest and aridest part (Alcaraz *et al.*, 1991).

Samples were collected from 16 sites, including monuments (12), calcareous rocks (3) and a new building (1) (Tab. 1), during the spring and summer of 1996 and 2003. Substrata colonized by the algal biofilms were limestone, sandstone and, in one case, concrete. Collections were made at sites where biofilms were visually detectable as black, green or grey microbial mats and crusts on/inside the stone. The samples were kept dry in plastic and paper bags in a cooler at 4°C in the Laboratory of Algology (Murcia University) before examination. Cultivation is usually necessary for detailed taxonomic studies of subaerial cyanophytes and algae, as the thalli of the microrganisms occurring in the biofilms are usually covered by large amounts of inorganic material in native preparations. The morphology of the species was therefore studied both from field-collected material and from cultivated specimens (subcultures and unialgal cultures). Part of the scraped field material was aseptically spread into test tubes and over the surface of Petri dishes containing BG11 medium (Rippka *et al.*, 1979), $BG11_0$ (Rippka, 1988) and BBM (Smith & Bold, 1966), either liquid or agarised. The test tubes and Petri dishes were incubated in constant conditions at 20ºC, 64.2 % humidity, 75 $\mu E\ m^{-2}\ s^{-1}$, 16:8 light:dark, in the laboratory of the S.A.C.E. (Servicio de Apoyo a las Ciencias Experimentales, Murcia University). After the first cultivation in Petri dishes (7-21 days of incubation), most of the species were examined and isolated into unialgal strains, which were used to support field observations in the determination of the species. Microscopic examinations were made with a stereomicroscope Olympus SZH and a microscope Olympus BH2. From the cultivated samples, 58 unialgal strains were isolated (Tab. 2); these are maintained in LAUM culture collection (Laboratory of Algology, Murcia University, Spain). Collected samples are preserved as dry material and the photographic documentation of the species recorded is available in the Laboratory of Algology, Murcia University (Spain).

The following publications were used for the identification of subaerial algae and cyanobateria: Frémy (1929), Geitler (1932), Pascher (1939), Desikachary (1959), Starmach (1966), Groover & Bold (1969), Ettl (1978), Komárek & Fott (1983), Anagnostidis & Komárek (1985), Hoffmann (1986), Komárek & Anagnostidis (1986), Anagnostidis & Komárek (1988), Ettl & Gärtner (1988), Komárek & Anagnostidis (1989), Hindák (1990), Round *et al.* (1990), Albertano & Kovacik (1994), Ettl & Gärtner (1995), Lokhorst (1996), Andreyeva (1998), Komárek & Anagnostidis (1998), Rifón-Lastra & Noguerol-Seoane (2001), John (2002) and Whitton (2002). Abbreviations for authorities follow Brummitt & Powell (1992).

RESULTS AND DISCUSSION

Floristic account

Overall, 47 epilithic and chasmoendolithic taxa were identified: 22 cyanobacteria (48%), 4 heterokontophytes (9%) and 20 chlorophytes (43%) (Tab. 3).

Table 2. Isolated algal and cyanophyte strains.

	Code of the strain	*Taxa*
1	MUL1/01A	***Tetracystis sarcinalis*** Schwarz
2	MUL2/01A	***Chlorella kessleri*** Fott & Nováková
3	MUL2/01B	***Chlorosarcinopsis*** cf. ***arenicola*** Groover & Bold
4	MUL2/01D	***Chlorella kessleri*** Fott & Nováková
5	MUL3/01A	***Chlorella vulgaris*** Beijerinck
6	MUL3/01B	***Chlorella*** sp.
7	MUL3/01C	***Chlorella vulgaris*** Beijerinck
8	MUL3/01D	***Chlorella*** sp.
9	FAB1/01A	***Ecdysichlamys obliqua*** G.S. West
10	FAB1/02A	***Diadesmis*** cf. ***contenta*** (Grunov) D.G. Mann
11	FAB1/02B	***Oocystis asymmetrica*** W. & G.S. West sensu Komáromy
12	FAB1/02C	***Chlorella kessleri*** Fott & Nováková
13	FAB1/02D	***Oocystis asymmetrica*** W. & G.S. West sensu Komáromy
14	FAB1/02E	***Chlorella vulgaris*** Beijerinck
15	FAB1/03A	***Xanthonema*** sp.
16	FAB1/03B	***Oocystis asymmetrica*** W. & G.S. West sensu Komáromy
17	FAB1/03C	***Diadesmis*** cf. ***contenta*** (Grunov) D.G. Mann
18	FAB1/03D	***Ecdysichlamys obliqua*** G.S. West
19	FAB1/03E	***Oocystis asymmetrica*** W. & G.S. West sensu Komáromy
20	FAB2/01A	***Klebsormidium flaccidum*** (Kützing) Silva, Mattox & Blackw.
21	FAB2/02B	***Klebsormidium flaccidum*** (Kützing) Silva, Mattox & Blackw.
22	FAB3/01A	***Oocystis asymmetrica*** W. & G.S. West sensu Komáromy
23	BES5/01A	***Chlorosarcinopsis*** cf. ***arenicola*** Groover & Bold
24	BES5/02A	***Chlorella kessleri*** Fott & Nováková
25	BES5/02B	***Chlorosarcinopsis*** cf. ***arenicola*** Groover & Bold
26	BES5/03A	***Chlorella kessleri*** Fott & Nováková
27	BES5/03B	***Chlorosarcinopsis*** cf. ***arenicola*** Groover & Bold
28	BES5/03C	***Chlorosarcinopsis*** sp.
29	BES11/03A	***Tetracystis sarcinalis*** Schwarz
30	BES11/04A	***Tetracystis sarcinalis*** Schwarz
31	BES12/02C	***Botrydiopsis*** sp.
32	BES14/01A	***Leptolyngbya*** sp. 2
33	LPT1/01C	***Chlorosarcinopsis*** sp.
34	LPT1/01E	***Stichococcus minutus*** Grintzesco & Péterfi
35	LPT1/03C	***Klebsormidium crenulatum*** (Kützing) Lokhorst
36	LPT3/01A	***Muriella terrestris*** J.B. Petersen
37	LPT3/01D	***Stichococcus bacillaris*** Nägeli
38	LPT3/02A	***Muriella terrestris*** J.B. Petersen
39	LPT3/02B	***Botrydiopsis*** sp.
40	LPT3/02C	***Heteropedia*** cf. ***simplex*** Pascher
41	LPT3/02D	***Stichococcus bacillaris*** Nägeli
42	LPT4/01A	***Chlorella kessleri*** Fott & Nováková
43	LPT4/01B	***Klebsormidium nitens*** (Menenghini in Kützing) Lokhorst
44	LPT4/01C	***Chlorella vulgaris*** Beijerinck
45	LPT4/01D	***Chlorella vulgaris*** Beijerinck
46	LPT4/02A	***Nautococcus terrestris*** P.A. Archibald
47	LPT5/01A	***Botrydiopsis*** sp.
48	LPT5/01B	***Muriella terrestris*** J.B. Petersen
49	LPT5/01C	***Muriella terrestris*** J.B. Petersen
50	LPT5/02A	***Botrydiopsis*** sp.
51	LPT5/02B	***Muriella terrestris*** J.B. Petersen
52	LPT7/01A	***Ecdysichlamys obliqua*** G.S. West
53	LPT8/01A	***Scenedesmus obtusiusculus*** Chodat
54	LPT8/02A	***Chlorella kessleri*** Fott & Nováková
55	LPT9/01A	***Scenedesmus obtusiusculus*** Chodat
56	LPT10/01A	***Chlorosarcinopsis*** cf. ***arenicola*** Groover & Bold
57	LPT11/01A	***Ecdysichlamys obliqua*** G.S. West
58	LPT11/02A	***Ecdysichlamys obliqua*** G.S. West

Table 3. List of species sampled and investigated from sites in SE Spain.
(* = first report for Spain)

Taxa	*Sampling sites*															
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
Pseudocapsa dubia							●		●							
**Cyanobacterium cedrorum*									●							
Synechocystis sp. type 1					●											
Synechocystis sp. type 2									●	●						
Aphanocapsa muscicola									●							
Chroococcidiopsis kashaii	●	●	●	●		●	●	●	●	●	●	●	●	●		●
Hyella balani															●	
Pseudanabaena sp.									●							●
Leptolyngbya sp. 1											●					●
Leptolyngbya sp. 2					●											
Leptolyngbya sp. 3																
Leptolyngbya nostocorum									●							●
Leptolyngbya sp. 4													●			
Leptolyngbya sp. 5									●							●
Leptolyngbya sp. 6															●	
Schizothrix friesii	●															
Phormidium autumnale												●	●			
Microcoleus vaginatus	●											●				
Scytonema julianum		●														
Tolypothrix byssoidea							●									
Calothrix fusca var. *crassa*													●			
Nostoc sphaericum	●						●				●					●
Botrydiopsis sp.												●				●
Heterothrix sp.					●								●			
Heteropedia cf. *simplex*																●
Hantzschia amphioxys													●			●
Diadesmis cf. *contenta*													●			
**Nautococcus terrestris*																●
**Tetracystis sarcinalis*		●														●
Trebouxia arboricola													●			
Myrmecia cf. *globosa*																●
Apatococcus lobatus		●	●									●				
Chlorosarcinopsis sp.										●					●	●
Chlorosarcinopsis cf. *arenicola*		●	●							●			●			●
**Ecdysichlamys obliqua*													●			●
**Oocystis asymmetrica*													●			
Muriella terrestris			●										●			●
Chlorella vulgaris	●		●										●			●
Chlorella sp.																●
**Chlorella kessleri*		●								●			●			●
Scenedesmus obtusiusculus																●
**Klebsormidium nitens*														●		●
Klebsormidium flaccidum										●			●			
**Klebsormidium crenulatum*																●
**Stichococcus allas*													●			
Stichococcus bacillaris		●								●			●	●		●
Stichococcus minutus																●

A description of the vegetative and reproductive morphology, with notes on autoecology and distribution, is reported in detail for the new or taxonomically uncertain taxa recorded in this study. Depending on habitat conditions, some species showed a considerable variation in morphology.

Determination of coccoid and some filamentous species, mainly belonging to the genera *Heterococcus*, *Chlorella* and *Klebsormidium*, was supported by morphological features in laboratory culture, in agreement with previous studies (Lokhorst, 1996; Kalina & Punčochárová, 1987). We could not identify with certainty some cyanobacteria, diatoms and green algae due to a lack of critical features or because the combination of morphological or ecological characters for these algae was not in agreement with any described species. Species recorded for the first time for Spain are marked by an asterisk.

CYANOPHYTA

***Pseudocapsa dubia* Erceg.** (Fig. 1)

This cyanophyte was identified at the sampling sites 7 and 9. In the structure of colonies and subcolonies it is similar to *P. maritima* Komárek, which has a different cell size (1-5 µm diam.). *P. dubia* is a typical subaerial species, reported from calcareous substrata (mainly caves) by Ercegović (1925) and Golubić (1967) in Croatia, by Palik (1938) in Hungary, by Starmach (1966) in High Tatras (Poland), by Skuja (1970) in Italy, by Asencio & Aboal (2000) in Spain and by Uher & Kovacik (2002a) in Slovakia.

*** *Cyanobacterium cedrorum* (Sauv.) Komárek *et* Anagn.** (Fig. 2)

Cells solitary or in pairs, pale blue-green, widely elliptic to spherical or subspherical, 4-7(10) × 2.5-5(6) µm.

This species was identified at the site 9. *C. cedrorum* is mainly reported from warm areas of the northern temperate zone and sporadically from subtropical and tropical countries (Komárek & Anagnostidis 1998). This is the first record of this species as biodeteriogen on monuments.

***Synechocystis* sp. 1** (Fig. 3)

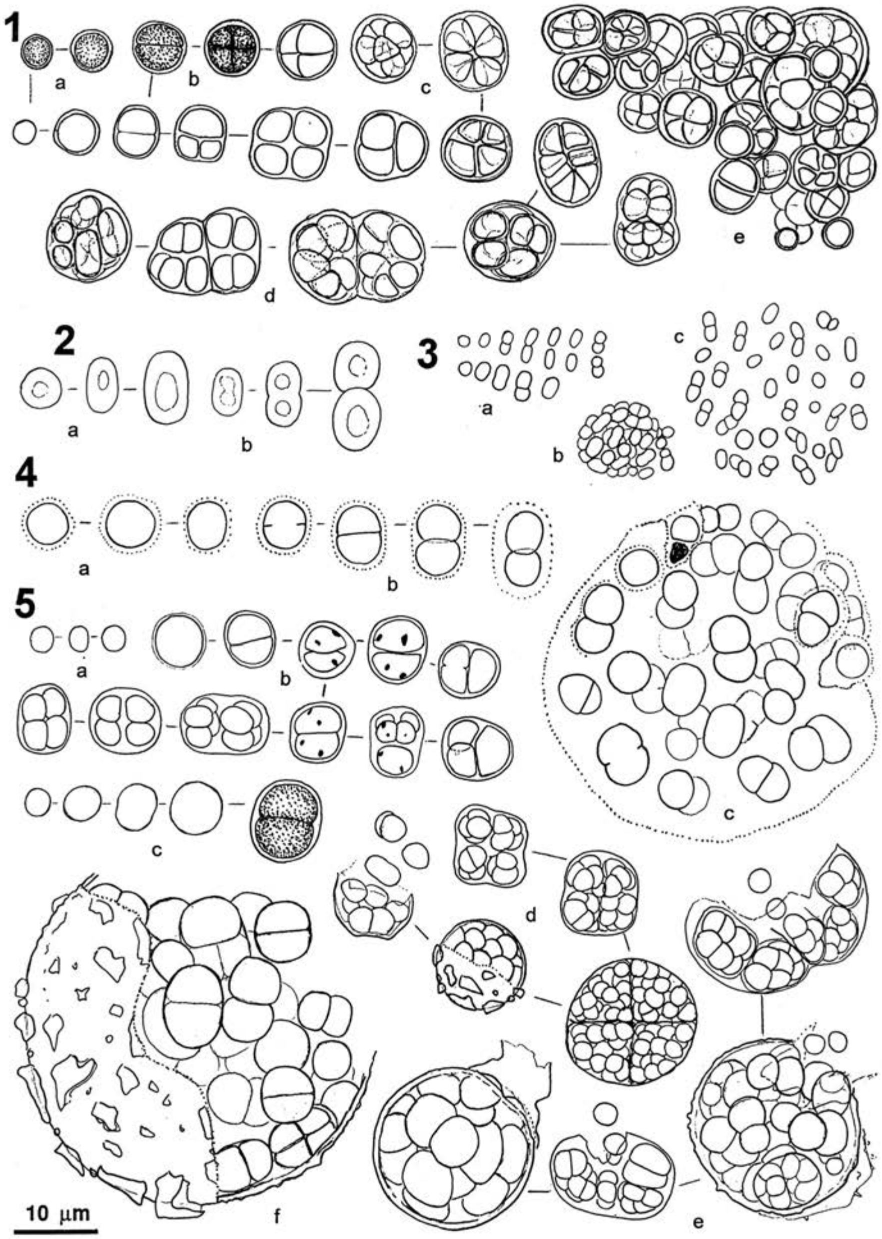
Cells solitary, in pairs or in small groups, elliptical, spherical or subspherical, olive- or pale blue-green, 1.2-2.5 × 1-2 µm.

This taxon was recorded at the site 5. Similar to *S. diplococca* (Pringsheim) Bourr. in size (1-1.4 µm diam.), it is, however, ecologically different from that species, which is known from fissures of rocks and stones influenced by steam exhalations of thermal volcanic fumaroles from Greece (Komárek & Anagnostidis 1998). Similar also to *Synechocystis pevalekii* Erceg., which is different in cell size (2.4-3.5 µm diam.).

***Synechocystis* sp. 2**

Cells solitary, globular, pale blue-green, 0.5-1 µm diam.

This taxon was recorded at sites 9 and 10. Similar in size and ecology to *S. primigenia* N.L.Gardner, which has cells 0.7-1 µm diam. *S. primigenia* was



Figs 1-5. **1**. *Pseudocapsa dubia*: *a* initial stages, *b* cell division in two planes, *c* cell division in three and more planes, radially oriented, *d* subcolonies of cells, *e* colony. **2**. *Cyanobacterium cedrorum*: *a* solitary cells, *b* cell division. **3**. *Synechocystis* sp.1: *a* cell division, *b* compact colony, *c* dispersed colony. **4**. *Aphanocapsa muscicola*: *a* initial cells with fine mucilage, *b* cell division, *c* mucilaginous colonies. **5**. *Chroococcidiopsis kashaii*: *a* initial endolithic stadia, *b* cell division, *c* initial epilithic stages, *d* endolithic cell colonies, *e, f* epilithic cell colonies.

described for material occurring on calcareous rocks in Puerto Rico (Komárek & Anagnostidis, 1998). This species has been recorded by Kapusta & Kovacik (2000) on monuments in Bratislava (Slovakia). The material from Murcia, however, cannot be referred with certainty to this species because of different cell shape (variable, not perfectly globular) and absence of a mucilaginous envelope, which was detected in only a few cells.

Aphanocapsa muscicola **(Menegh.) Wille** (Fig. 4)

This species was identified at the site 9. Species of *Aphanocapsa* are known from lithic monument habitats (Mannino, 1991; Tomaselli *et al.*, 2000). Typical subaerial species, on wet rocks, wood and soil; widespread in temperate zones (Komárek & Anagnostidis, 1998). *A. muscicola* was reported for caves and aquatic habitats from Southeastern Spain (Aboal, 1988; Asencio & Aboal, 2000).

Chroococcidiopsis kashaii **Friedmann** (Fig. 5)

This species was identified at the sites 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 16. It was described by Friedmann (1961) from caves in Israel. It is a very common species in the Region of Murcia. It was cultivated in subcultures, but we did not succeed in isolation into unialgal strains. In subcultures it produced smaller colonies than in natural conditions, only 8-10(15) µm diam.; granules were present in the cells and the colour was olive-green (not blue-green). Species of the genus *Chroococcidiopsis* are important biodeteriogens of monuments. Asencio & Aboal (2000) recorded *C. doonensis* R.B.Singh and *C. kashaii*. *C. kashaii* was also investigated by Abdelahad (1985) from underground in Italy. The species also grows epilithically, producing larger colonies and bigger cells (6-7 µm diam.) than in the case of the chasmoendolithic forms (3-5 µm diam.). The same situation is observed in culture, where the cells become smaller (2.5-3 µm diam.).

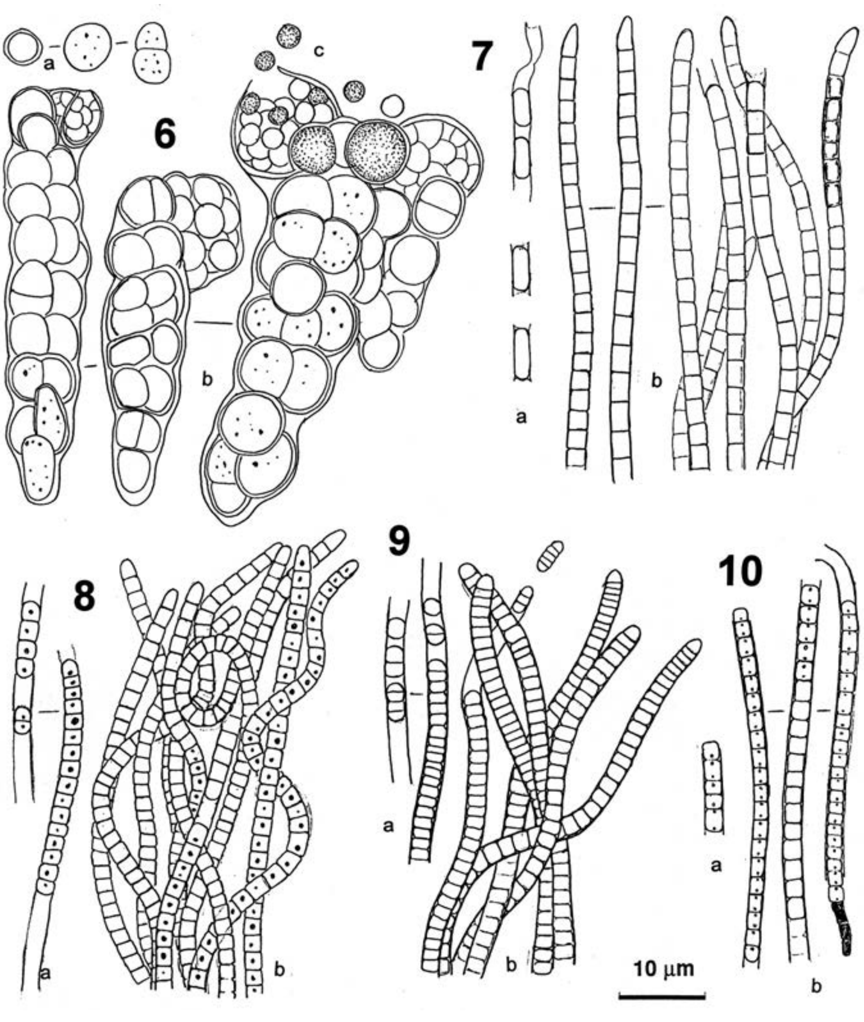
Hyella balani **Lehmann** (Fig. 6)

This is a marine species, growing on limestone coasts mainly in Mediterranean Sea (Komárek & Anagnostidis, 1998). It was sampled from limestone in the cavity of a salt chalk spring, the site 15 (conductivity of the salt water C = 20500 µS cm^{-1}, t = 24.8°C.). In epilithic microbiotopes the alga produces smaller cells (2-4 µm diam.), as gloeocapsoid forms, than in chasmoendolithic conditions (6-8 µm diam.). In the genus *Hyella*, *H. fontana* was reported as a biodeteriogen by Giaccone *et al.* (1976) from monuments of Italy, by Mannino (1991) from monuments of Mediterranean Europe and by Ortega-Calvo *et al.* (1993) from the Casa degli Aurighi and Ostia Scavi in Italy.

Pseudanabaena **sp.** (Fig. 7)

Thallus dark green to black, formed by filaments joined in small colonies, sometimes fragmented into solitary cells; cells cylindrical, 2 (2.5)-times longer than wide, with a clearly observable dense zone of thylakoids (darker strips by the cell wall) in chromoplasma, constricted at cross walls, 3-7.5 × 3-3.5 µm. Trichomes are simple, without branching, enclosed in a very thin sheath; apical cells without calyptra, conical with rounded apex.

This taxon was recorded at the sites 9 and 16. It was found in association with *Leptolyngbya* sp. 5. Close in size and habit to other genera (e.g. *Phormidium* and *Leptolyngbya*), but the characters of the trichome are typical of species of



Figs 6-10. **6**. *Hyella balani*: *a* initial cells, *b* endolithic colonies, *c* baeocytes. **7**. *Pseudanabaena* sp.: *a* cell fragmentation of trichome, *b* filaments. **8**. *Leptolyngbya* sp. 1: *a* granulated hormogonia, *b* filaments. **9**. *Leptolyngbya* sp. 2: *a* hormogonia, *b* filaments. **10**. *Leptolyngbya* sp. 3: *a* hormogonium, granulated by crosswalls, *b* filaments.

Pseudanabaena (Anagnostidis & Komárek, 1988). It could not be identified with certainty because width of trichomes is not in agreement with any species of *Pseudanabaena*.

***Leptolyngbya* sp. 1** (Fig. 8)

Thallus blue-green to dark green; trichomes straight, simple, densely parallel, flexuous, with a very thin sheath; cells isodiametric, mostly granulated in the centre, sometimes near the cross walls, slightly constricted at the cross walls; cells 2-3 µm diam.; apical cells without calyptra, widely conical, with rounded apex.

This taxon was recorded at the sites 11 and 16. It is similar to *L. perforans* (Erceg.) Anagn. *et* Komárek, but could not be attributed with certainty to this species because of different cell size and presence of central granules. *Leptolyngbya* sp. 1 forms chasmoendolithic associations with *Chroococcidiopsis kashaii* and *Hantzschia amphioxys*. *L. perforans* has been recorded by Asencio & Aboal (2000) for caves in the north of Murcia; it was also reported by Geitler (1932) as endolithic or in soils. Starmach (1966) mentioned this organism (as *Schizothrix perforans*) as a species with broad distribution, occurring in springs, lakes, on sandy substrates, stones, calcareous soils and shells. Pentecost (1992) mentioned it for calcareous springs.

***Leptolyngbya* sp. 2** (Fig. 9)

Thallus olive-green to brownish green; trichomes simple, slightly flexuous, straight, erected, enclosed in a thin sheath; cells isodiametric or wider than longer, (1)1.5-2 × 2 μm, devoid of granulations, slightly constricted at the cross walls. Apical cells without calyptra, widely conical. Reproduction takes place by few-celled hormogonia.

It was found at the site 5. This cyanophyte produced chasmoendolithic associations with *Chroococcidiopsis kashaii.* The morphological features were close to the characters of *L. foveolarum* (Rabenhorst ex Gomont) Anagn. *et* Komárek. It could not be identified with certainty because of different autecology and different characters, such as widely conical apical cells. *L. foveolarum* was reported growing on soils and rocks in shallow streams (Desikachary, 1959, as *Phormidium foveolarum* (Mont.) Gomont). Anagnostidis *et al.* (1992) mentioned it as a biodeteriogen of the marbles of the Parthenon and Propylaea (Acropolis, Athens).

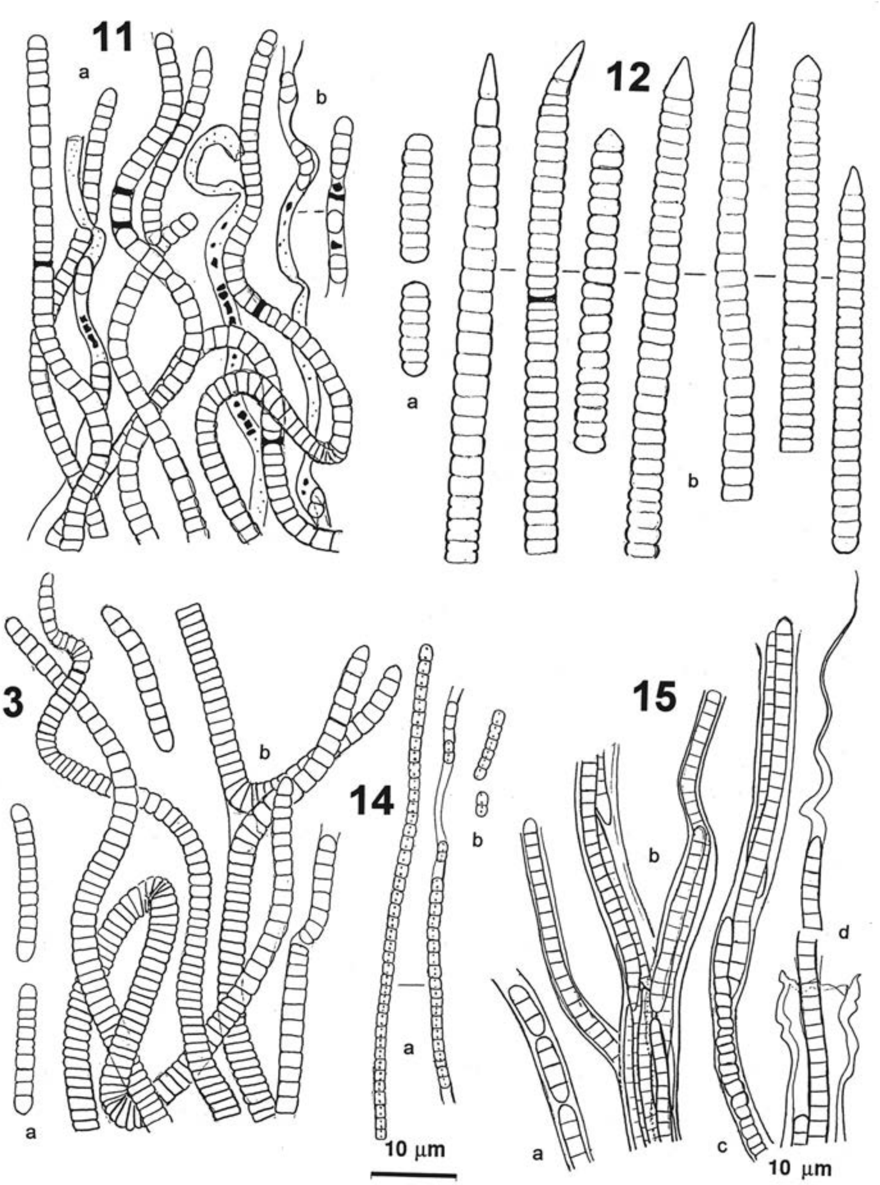
***Leptolyngbya* sp. 3** (Fig. 10)

Thallus blue-green, mucilaginous; trichomes simple, flexuous, without branching, with a very thin sheath, frequently fragmented; cells isodiametric, granulated and slightly constricted at the cross walls, 1.5-2 μm diam.; apical cells without calyptra, widely rounded.

This cyanobacterium was recorded at the site 9. In cell size it is similar to *L. fragilis* (Menegh.) Anagn. *et* Komárek, but for the presence of sheath and cell size the morphology of this organism also overlaps with *L. amplivaginata* (Van Goor) Anagn. *et* Komárek and *L. foveolarum* (see Albertano & Kovacik, 1994). The untypical combination of cell size and presence of sheath did not allow unambiguous identification. *L. fragilis* was recorded by Kapusta & Kovacik (2000) from monuments of Bratislava (Slovakia), by Leitao *et al.* (1996) from aeroplankton of Coimbra (Portugal) and by Ortega-Calvo *et al.* (1993) as *Phormidium fragile* (Menegh.) Gomont from Italian and Spanish monuments. *L. fragilis* is a controversial taxon, regarded as a cosmopolitan and salinity-indicating species.

***Leptolyngbya nostocorum* (Bornet ex Gomont) Anagn. *et* Komárek** (Fig. 11)

This species was recorded at the site 16. Endogloeic species, occurring in the mucilage of colonies of *Tetracystis* and *Microcoleus vaginatus*. This species was mentioned as biodeteriogen by Kapusta & Kovacik (2000) on monuments in Bratislava and by Mannino (1991) on monuments in Italy. The populations of this species reproduce by fragmentation of trichome by numerous necridic cells, which has not been yet reported.



Figs 11-15. **11**. *Leptolyngbya nostocorum*: *a* filaments with necridic cells, *b* hormogonia. **12**. *Leptolyngbya* sp. 4: *a* hormogonia, *b* filaments with conical cells. **13**. *Leptolyngbya* sp. 5: *a* hormogonia, *b* colony with false-branching filaments. **14**. *Leptolyngbya* sp. 6: *a* filaments, granulated by crosswalls, *b* hormogonia. **15.** *Schizothrix friesii, a* hormogonia, *b* filaments in common sheats, *c-d* apical parts of filaments. Left scale is applied to Figs 11-14; right scale is applied to Fig. 15.

Leptolyngbya sp. 4 (Fig. 12)

Thallus dark green; trichomes simple, flexuous, straight, devoid of sheath; cells mostly shorter than broad or isodiametric, 2-4 × (3) 3.5-4 µm, nongranulated, constricted at the cross walls; apical cells sharply conical, without calyptra. Fragmentation by necridic cells.

This species formed mucilaginous brownish-green carbonated patinas in association with *Chlorosarcinopsis* and *Microcoleus*; it was recorded at the site 13, a concrete building. The morphological features are in agreement with the characteristics of *L. boryana* (Gomont) Anagn. *et* Komárek (Albertano & Kovacik, 1994), but a sure identification was not possible because of the lack of sheath, fragmentation by necridic cells and conical apical cells.

Leptolyngbya sp. 5 (Fig. 13)

Thallus brownish-green to brown; trichomes in colonies, dense, fragile, branched, with sheath; cells slightly constricted at the cross walls, (2)2.5-3 µm diam., isodiametric in the apical parts of the trichomes, shorter than broad and wider in the middle of the trichomes, (1)1.5-2 × 3 µm. Apical cells rounded, usually smaller than the vegetative cells of trichome, without calyptra. Reproduction by hormogonia.

This species formed mucilaginous green growths with species of *Pseudanabaena* and *Tetracystis* and with *Chroococcidiopsis kashaii*; it was recorded at the sites 9 and 16. *L. boryana* (Gomont) Anagn. *et* Komárek is similar to this species (Roussomoustakaki, 1983), but other features of the thallus (composition of trichomes, branching) are closer to *Plectonema purpureum* Gomont, which has cells 1-2.5 × 3 µm (Geitler, 1932). *L. boryana* is known as a biodeteriogen from monuments of Athens (Anagnostidis *et al.*, 1992), from monuments of Italy (Ortega-Calvo *et al.*, 1991) and from the cathedrals of Salamanca and Toledo, Spain (Ortega-Calvo *et al.*, 1993).

Leptolyngbya sp. 6 (Fig. 14)

Thallus mucilaginous, dark-green; trichomes in elliptic, compressed colonies, with very thin sheath, frequently fragmented; cells granulated, slightly constricted at cross walls, isodiametric, 1.5-1.6 µm diam. Apical cell widely rounded, without calyptra.

This cyanobacterium was recorded at the site 15. It is similar to *L. fragilis* in size and to *L. tenuis* (Menegh.) Anagn. *et* Komárek in the organization of the trichomes (apical cell, sheats) and in ecological features. This taxon could not be identified with certainty because of the untypical combination of habit of thallus and cell size. Ortega-Calvo *et al.* (1991, 1993) reported *L. tenuis* (as *Phormidium tenue* (Menegh.) Gomont) on marble, granite and sandstone of Spanish monuments. Rindi & Guiry (2003) recorded *P.* cf. *tenue* from subaerial habitats of Galway City (Ireland) and Darienko & Hoffmann (2003) recorded *L. tenuis* from monuments of Olivia City (Ukraine).

Schizothrix friesii (C. Agardh) Gomont (Fig. 15)

This cyanobacterium was identified at the site 1. It formed associations with *Nostoc, Microcoleus, Chroococcidiopsis* and *Stichococcus*. It is a widely known biodeteriogen of monuments, reported from subaerial habitats (Wee & Lee, 1980; Wee, 1982; Aboal, 1988; Lee & Eggleston, 1989; Asencio & Aboal, 2000). Frémy (1929), Geitler (1932) and Starmach (1966) reported this species from rocks, bark of tree near the ground and mosses.

Phormidium autumnale **Gomont** (Fig. 16)

This taxon was identified at the sites 12 and 13. It is a very common cosmopolitan species of terrestial habitats. This species is morphologicaly very similar to *P. uncinatum* (C. Agardh) Gomont. *P. autumnale* was mentioned by Kapusta & Kovacik (2000) from monuments in Bratislava (Slovakia), by Ortega-Calvo *et al.* (1991, 1993) from biodeteriorated buildings in Spain, by Wasser *et al.* (1988) from biodeteriorated buildings in Russia, by Darienko & Hoffmann (2003) for bricks of monuments in Kyiv (Ukraine) and by Rindi & Guiry (2003) for bases of urban walls in Galway City (Ireland). Aboal (1988) reported it as a common species in southeastern Spain.

Microcoleus vaginatus **(Vaucher) Gomont** (Fig. 17)

This cyanobacterium was identified at the sites 1 and 12. It is known mainly from soils (Geitler, 1932; Desikachary, 1959). It was reported also as biodeteriogen by Mannino (1991) from the Mediterranean area, by Ortega-Calvo *et al.* (1991, 1993) from monuments of Spain and Italy, by Schlichting (1975) from monuments of Ireland, and by Aboal (1988) from subaerial habitats in South-Eastern Spain.

Scytonema julianum **(Kütz.) Menegh.** (Fig. 18)

This species was identified at the site 2. It has often been placed in synonymy with *Scytonema hofmanni* Bornet *et* Flahault. Geitler, however, (1932) recognised the distinctness of *S. julianum*. Sandstone is the typical substratum of this species, which is mentioned as biodeteriogen by Andreyeva (1998) from buildings in Russia, by Ariño *et al.* (1997) from roman tombs in Spain and by Mannino (1991) from biodeteriorated buildings in Italy. Reported also from caves of SE Spain by Aboal *et al.* (1994).

Tolypothrix byssoidea **(Hassall) Kirchner** (Fig. 19)

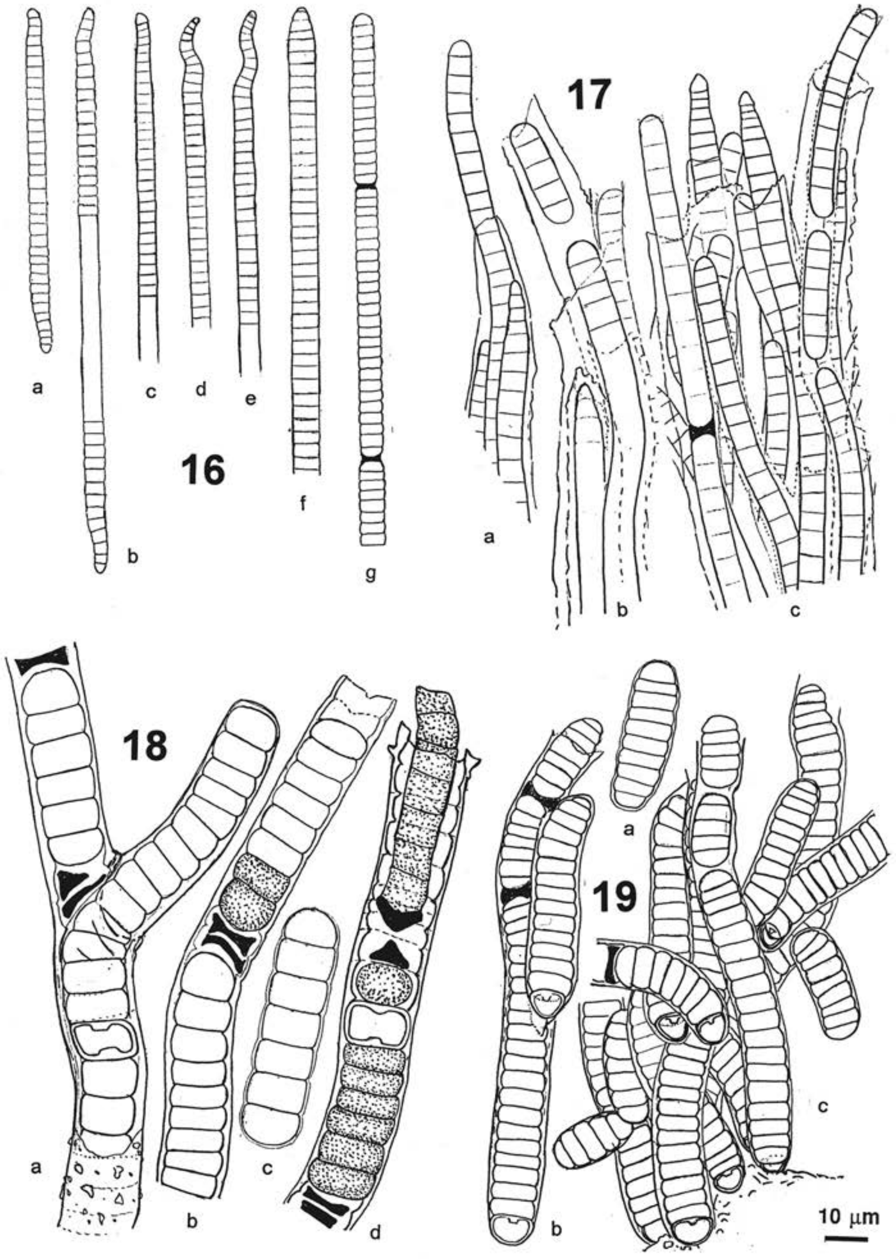
This species was identified at the site 7. It is known from tree trunks and moist soil (Desikachary 1959), but also as biodeteriogen of buildings (Grant, 1982; Mannino, 1991; Ortega-Calvo *et al.*, 1991, 1993; Rindi & Guiry, 2003). It was found in association with the moss *Tortula muralis* J. Hedwig.

Calothrix fusca **var.** ***crassa*** **Rao** (Fig. 20)

This cyanophyte was identified at the site 13. It was originally described from a pond in the city of Dharbhanga, in Bihar province, India (Desikachary, 1959). Our material differs from the original description in the number of heterocytes and in the constrictions at the cross-walls. It is also similar to *Calothrix parietina* Thuret ex Bornet *et* Flahault, which has intercalary heterocytes, single or adjacent. *C. parietina* occurs on surfaces of submerged plants and rocks (Desikachary, 1959; Geitler, 1932) and is the most common species of *Calothrix* on monuments, recorded by Giaccone *et al.* (1976), Lee & Eggleston (1969), Ortega-Calvo *et al.* (1991), Turian (1979), Wee (1982), Wee & Lee (1980) and Rindi & Guiry (2003). *C. fusca* was mentioned as biodeteriogen of buildings by Mannino (1991) and Ortega-Calvo *et al.* (1991).

Nostoc sphaericum **Vaucher ex Bornet** ***et*** **Flahault** (Fig. 21)

This cyanobacterium was identified at the sites 1, 7, 11 and 16. It has been reported from moist soil and rocks in Bombay, Calcutta and Courtallum (Desikachary, 1959), in symbiosis with hornworts and liverworts (Geitler, 1932)



Figs 16-19. **16**. *Phormidium autumnale*: *a* hormogonium, *b-f* trichomes, *g* fragmentation by necridic cells. **17**. *Microcoleus vaginatus*: *a-b* apical parts of colonies, *c* colony of trichomes in common sheaths. **18**. *Scytonema julianum*: *a* false branching, *b* fragmentation by necridic cells, *c* hormogonium, *d* filament with heterocyte. **19**. *Tolypothrix byssoidea*: *a* hormogonium, *b* filament with basal heterocyte, *c* colony of filaments.

and as biodeteriogen by Aboal (1988), Asencio & Aboal (2000), Mannino (1991), Schlichting (1975) and Rindi & Guiry (2003). It grows both epilithically and chasmoendolithically and frequently shows a larger cells size (5-8 µm diam.), thicker sheat and smaller colonies in the chasmoendolithic form (2.5-5 µm diam.).

XANTHOPHYCEAE

***Botrydiopsis* sp.** (Fig. 22)

Cells single, spherical, 8-16 (20) µm diam.; mature cells with thick cell wall, with well observable double-layers. Chloroplasts numerous, first polygonal, then oval, rounded or spindle-shaped, parietal, with no clear pyrenoid. Reproduction by autospores produced at the periphery of the sporangium, 4-8 µm diam.; sporangium 16-35 µm diam.

This alga was recorded at the sites 5, 12 and 16. The specimens were similar to *Botrydiopsis arhiza* Borzi in size, but could not be identified with certainty because of lack of zoospores. Species of *Botrydiopsis* are known mostly from soil and subaerial habitats (Ettl, 1978). The strains isolated grew very well in BBM.

***Xanthonema* sp.** (Fig. 23)

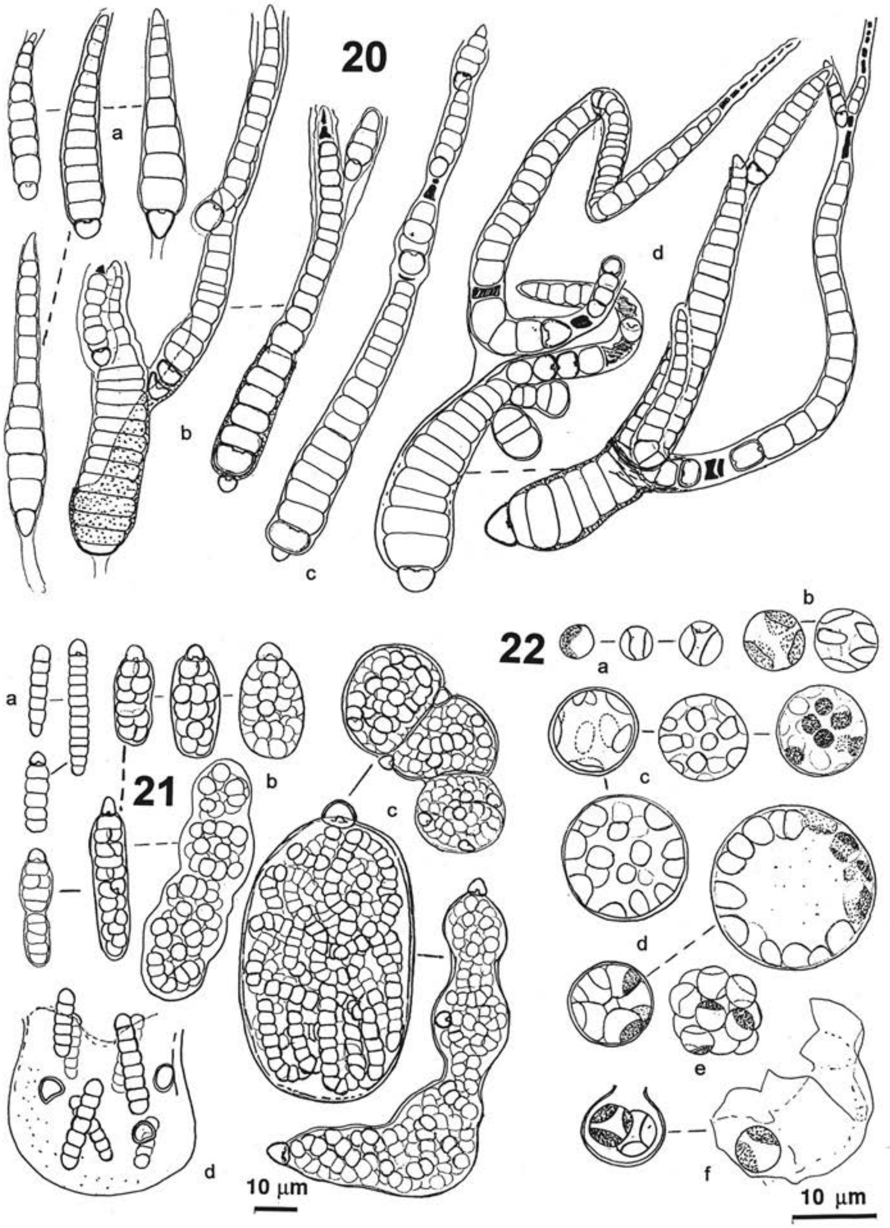
Thallus with very high morphological variability; cells either solitary, in 4-celled filaments or up to 24-78 in one trichome (in culture, BBM medium). Cells cylindrical, subspherical, rarely spherical, 8-10 × 10-20(35) µm, with one or two parietal chloroplasts; filaments very fragile, very well visible heterogenous structure of cell-walls (mostly in old cells). Spores (akinetes) in groups, sometimes very long, 10 × 20(35) µm, with 8 rounded chloroplasts

This taxon was recorded at the site 13. It could not be identified with certainty for the lack of zoospores and because of ambiguity in the combination of several characters, such as habit of trichomes, shape of chloroplast and production of untypical akinetes. It formed associations with *Chlorosarcinopsis, Chlorella, Klebsormidium, Phormidium* and *Stichococcus* on the concrete wall (site 13). *X. ulotrichoides* (Pascher) P.C. Silva was mentioned as biodeteriogen of monuments by Ortega-Calvo (1991) and by Schlichting (1975). *X. exile* (Klebs) P.C. Silva was reported by Asencio & Aboal (2000) for cave environments in the region of Murcia and by Darienko & Hoffmann (2003) from monuments of Ukraine, where other xanthophycean species, such as *X. hormidioides* (Vischer) Silva and *Heterococcus pleurococcoides* Pitschmann, were also found.

***Heteropedia* cf. *simplex* Pascher** (Fig. 24)

Thallus green to yellow-green, with pseudofilamentous and pseudoparenchymatous habit; vegetative cells spherical, subspherical or elongated, 6-15 µm diam., usually with one chloroplast (two chloroplasts immediately before cell division), parietal, urn-shaped; sporangia produced at the end of pseudofilaments 10-18(22) µm diam., autospores 2.5-5 µm diam.

This taxon was recorded at the site 16. It could not be identified with certainty for the lack of zoospores and production of autospores. The genus *Heteropedia* forms typical small pseudoparenchymous thalli with elongated, widened apical cells (Ettl & Gärtner, 1995). *H. simplex* Pascher and *H. polychloris* Pascher are reported as subaerial algae (Pascher, 1939).



Figs 20-22. **20**. *Calothrix fusca* var. *crassa*: *a* initial stages, *b* formation of false branches, *c* serial fragmentation of filament, *d* colonies of filaments. **21**. *Nostoc sphaericum*: *a* hormogonia, *b* initial stadia, *c* adult mucilaginous sphaerical colonies, *d* ruptured colony. **22**. *Botrydiopsis* sp.: *a* autospores, *b* young cells, *c* adult cells, *d* autosporangia, *e* autospores, *f* ruptured autosporangia. Left scale is applied to Figs 20-21; right scale is applied to Fig. 22.

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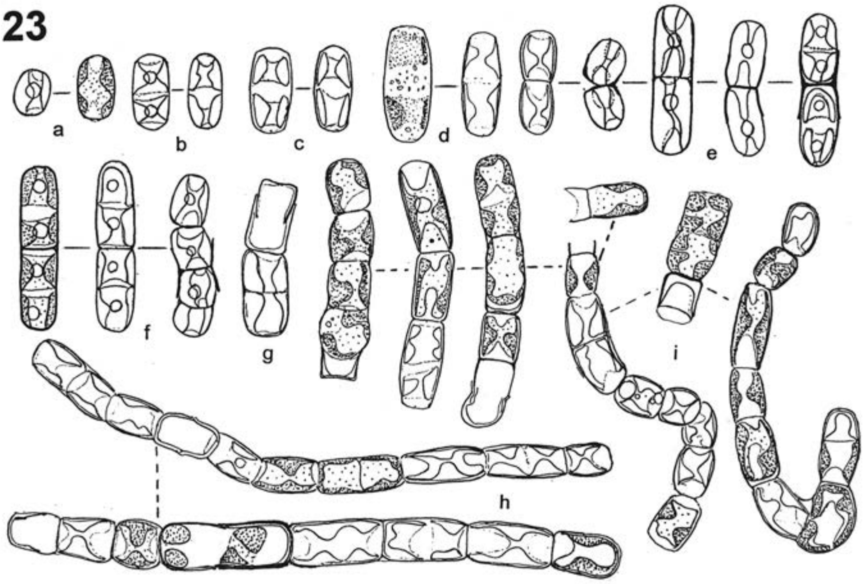

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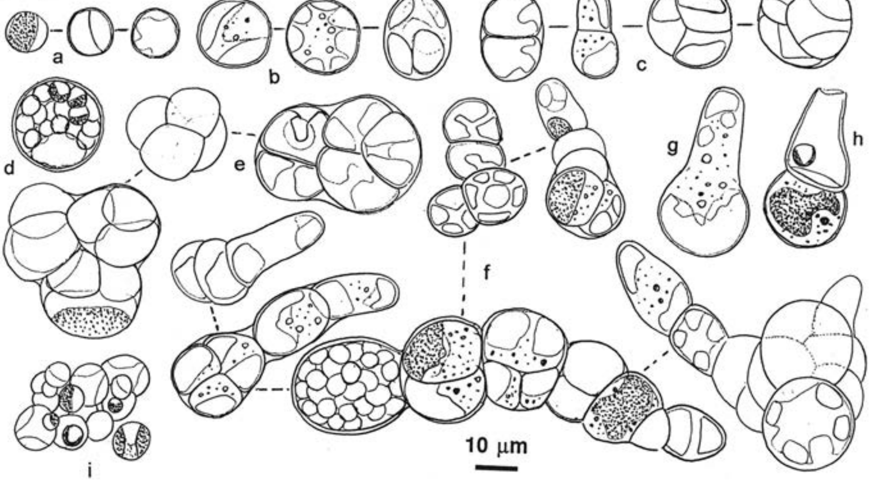


Figs 23-24. **23**. *Xanthonema* sp.: *a* unicell stages with one chromatophore, *b, c* unicellular stages with two chromatophores, *d* unicellular forms of maximal size, *e* cell division, *f* four-celled trichomes, *g* trichome with empty cells, heterogenic cell walls, *h* filaments, *i* fragmentation. **24**. *Heteropedia* cf. *simplex*: *a* autospores with one chromatophore, *b* young cells, *c* initial cell colonies, *d* unicelled autosporangium, *e* young colonies, *f* pseudofilaments, *g* apical prolonged cell, *h* empty autosporangium, *i* aggregated autospores.

BACILLARIOPHYCEAE

***Hantzschia amphioxys* (Ehrenb.) Grunow in Cleve *et* Grunow**

This alga was identified at the sites 13 and 16. It is a very common species of subaerial habitats. It was reported as a biodeteriogen of monuments by Altieri *et al.* (1993), Asencio & Aboal (2000), Kapusta & Kovacik (2000), Ortega-Calvo *et al.* (1991, 1993 a), Darienko & Hoffmann (2003) and Wasser *et al.* (1988).

***Diadesmis* cf. *contenta* (Grunow) D.G. Mann**

Cells 7-15 × 2-3 µm, with 36 striae per 10 µm.

This taxon was recorded at the site 16. It grew very well in BBM. It could not be identified with certainty because of differences in some characters, such as a narrow axial area and unclear raphe. *D. contenta* is a common subaerial species and has been reported from the region of Murcia by Asencio & Aboal (2000).

CHLOROPHYTA

*** *Nautococcus terrestris* Archibald** (Fig. 25)

Cells solitary or in 4-celled colonies, spherical, ovate, obovate or irregularly shaped, 8-14 µm diam. in young cultures, up to 20 µm in older cultures; polar caps produced by the cell walls. Chloroplast massive, lobed. Reproduction by autospores.

This taxon was identified at the site 16. This species was grown on BBM solid medium and flagellate stages were not observed. *N. terrestris* was originally isolated from soil in Fredericksburg, Texas (USA) and described from the cultures UTEX 1794 and CCAP 53/3 (Ettl & Gärtner, 1988).

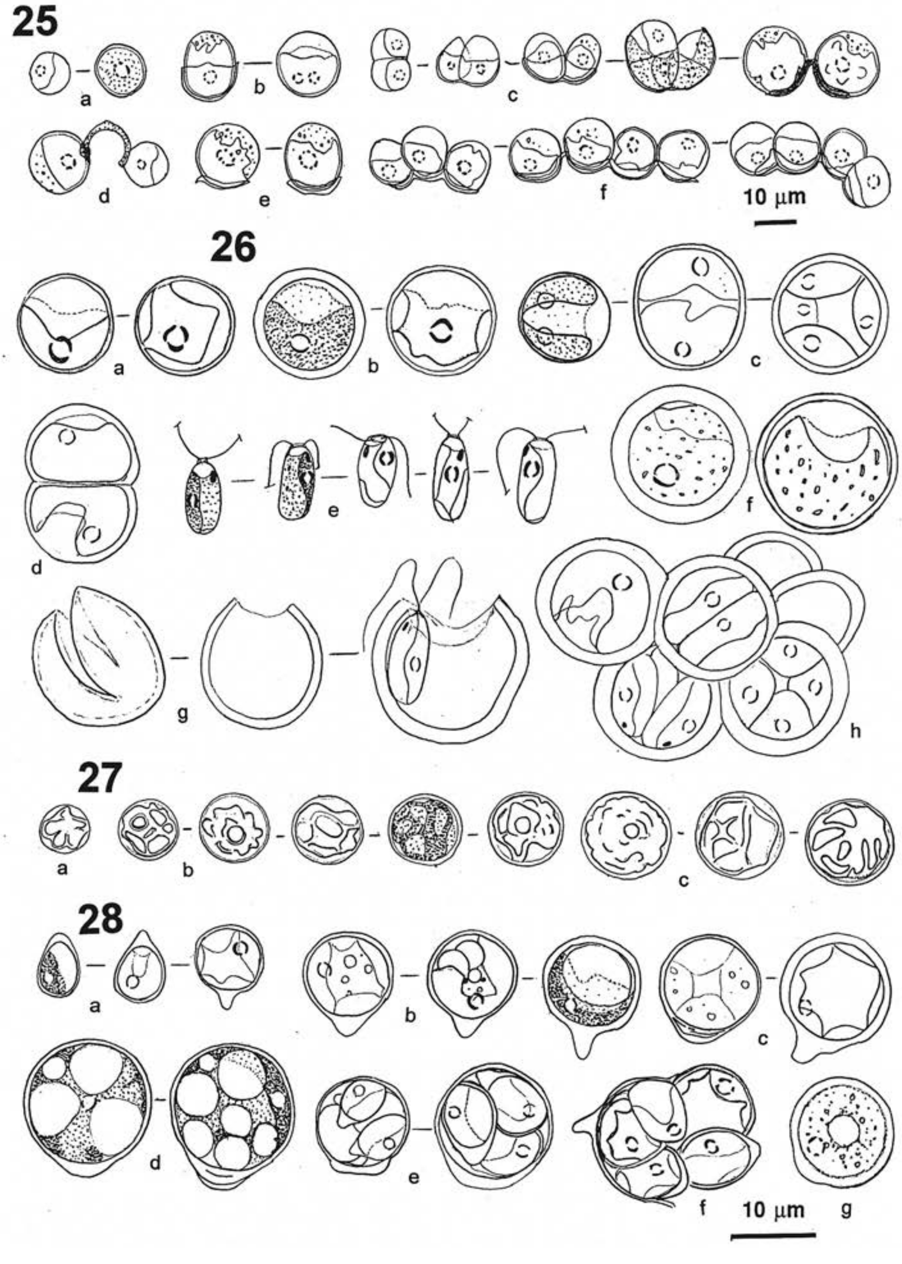
*** *Tetracystis sarcinalis* Schwarz** (Fig. 26)

Cells in mucilaginous colonies, dividing to produce tetrads; the wall of the original mother cell often includes cells up to the 3rd generation. Vegetative cells (8)10-12 µm diam., with one parietal chloroplast, urn-shaped and perforated in old cells. Zoospores with a stigma and a parietal chloroplast, 8-10 × 3-4 µm.

This taxon was identified at the sites 2 and 16. This species was originally described from soils of Dalmatia (Croatia). Species of *Tetracystis* are not frequently reported as biodeteriogens of monuments. *T. tetraspora* (Arce & Bold) Brown & Bold is mentioned by Darienko & Hoffmann (2003) from monuments of Ukraine.

***Trebouxia arboricola* De Puymaly** (Fig. 27)

This species was identified at the site 13. It is an epiphytic or epilithic species, known from monuments (Kapusta & Kováčik, 2000; Wasser *et al.*, 1988). *T. decolorans* Ahmadjian was reported by Ortega-Calvo *et al.* (1991, 1993) as biodeteriogen of monuments in Spain and Italy; *T.* cf. *arboricola* was reported by Rindi & Guiry (2003) from Galway City (Ireland). *T. schoumanii* Hildret *et* Ahmadjan was mentioned from monuments of Olivia (Ukraine) by Darienko & Hoffmann (2003).



Figs 25-28. **25**. *Nautococcus terrestris*: *a* spherical initial cells, *b* adult cells, *c* cell division, *d* daughter cells connected with rest of the envelope, *e* adult cells with cap (rest of mother cell wall), *f* cell colonies connected with mother cell wall. **26**. *Tetracystis sarcinalis*: *a* young cells, *b* adult cells with mucilage envelope, *c* zoosporangia, *d* cell division, *e* zoospores, *f* old cells, *g* empty zoosporangia, *h* colony. **27**. *Trebouxia arboricola, a* young cell with parietal chloroplast, *b* adult cells with axial, stellate chloroplast, *c* old cells. **28**. *Myrmecia* cf. *globosa*: *a* autospores, *b* young vacuolized cells, *c, d* adult vacuolized cells, *e* autosporangia, *f* ruptured autosporangium, *g* old cell. Top scale is applied to Fig. 25; bottom scale is applied to Figs 26-28.

***Myrmecia* cf. *globosa* Printz** (Fig. 28)

Cells solitary or in small groups, spherical, oval or obovate, 6-14(18) µm diam.; cell wall up to 2 µm wide, with a conical papilla at one pole of the cell; chloroplast urn-shaped, covering the majority of cell wall; autospores elliptic, 4 µm diam.

This alga was recorded at the site 16 and formed brown colonies on BBM agar plates. In habit it is similar to *M. globosa*, but could not be identified with certainty because of different autecology and cell size (see Ettl & Gärtner, 1995). Three species of *Myrmecia* have been reported as biodeteriogens: *M. biatorellae* (Tschermak-Woess *et* Plessl) J.B. Petersen from the aeroplankton of Portugal (Leitao *et al.*, 1996) and from monuments of Ukraine (Darienko & Hoffmann, 2003), and *M. bisecta* Reisigl and *M. incisa* Reisigl from monuments of Kyiv and Olivia in Ukraine (Darienko & Hoffmann, 2003).

***Apatococcus lobatus* (Chodat) J.B. Petersen** (Fig. 29)

This taxon was identified at the sites 2, 3 and 12. It is a common subaerial species. It was mentioned as biodeteriogen of monuments by Kapusta & Kovacik (2000) for Bratislava (Slovakia), by Lee & Eggleston (1989) from the aeroplankton of USA, by Ortega-Calvo (1991, 1993) for Italy and Spain and from subaerial habitats in Galway City (Ireland) by Rindi & Guiry (2003).

***Chlorosarcinopsis* sp.** (Fig. 30)

Sarcinoid colonies, tetrahedrically arranged, usually formed by more than 16 cells; cells oval to subglobular, 2.5-3(4) µm diam.; chloroplast cup-shaped, parietal, sometimes covering the whole cell wall; pyrenoid not clear. Zoospores not observed.

This taxon was recorded at the sites 9, 15 and 16. It formed associations with species of *Leptolyngbya*. It is similar to *Chlorosarcinopsis minor* (Gerneck) Herndon, which was reported from buildings in the USA by Brook (1968). It could not be identified with certainty because of the lack of zoospores.

***Chlorosarcinopsis* cf. *arenicola* Groover *et* Bold** (Fig. 31)

Colonies pocket-like, very dense, with sarcinoid arrangement; cells globular, subglobular or irregularly shaped, 6-8(10) µm diam.; chloroplast cup-shaped, parietal, with pyrenoid. Zoospores oval, with stigma and cup-shaped chloroplast, (4)5-6 µm diam.

This taxon was recorded at the sites 2, 3, 9, 13 and 16. It could not be identified with certainty because of differences in the morphology of the zoospores. It is very common in subaerial habitats of Murcia, where it was reported by Asencio & Aboal (2000) from caves. This alga is closely similar to *C. variabilis* Trainor *et* Hilton, which has larger zoospores, 7.5-15.8 × 3-4 µm (Ettl & Gärtner, 1995). *C. arenicola* was described by Groover & Bold (1969) from sandstone soil. *C. gelatinosa* Chantanachat *et* Bold was mentioned by Kapusta & Kovacik (2000) for monuments of Bratislava. *C. negevensis* Friedmann & Ocampo-Paus was mentioned for monuments of Ukraine (Darienko & Hoffmann, 2003).

* ***Ecdysichlamys obliqua* G.S.West** (Fig. 32)

Cells solitary, in groups or in small colonies enclosed in the mother cell wall. Cells spindle-shaped, elliptical or oval, asymmetrical 6.5-10 × 5-10 µm; chloroplast parietal, cup-shaped, with pyrenoid; cell wall thick, smooth, colourless, with

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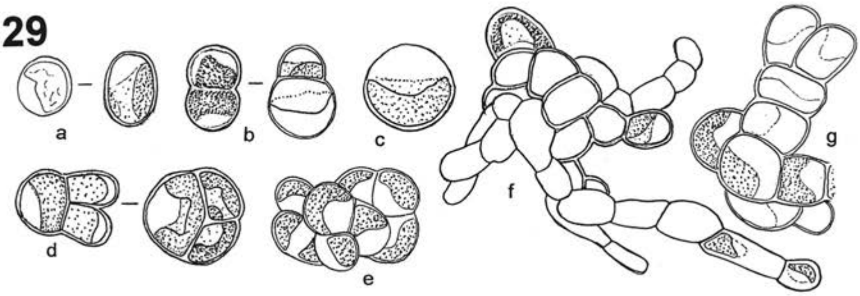

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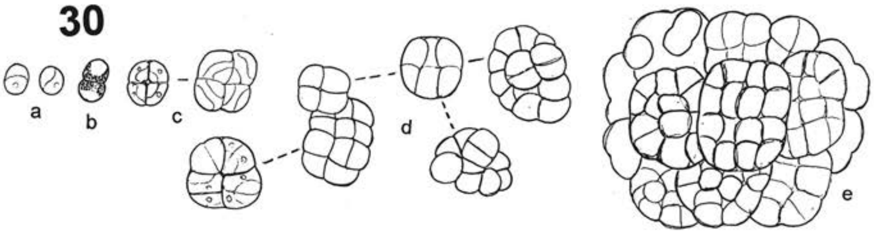

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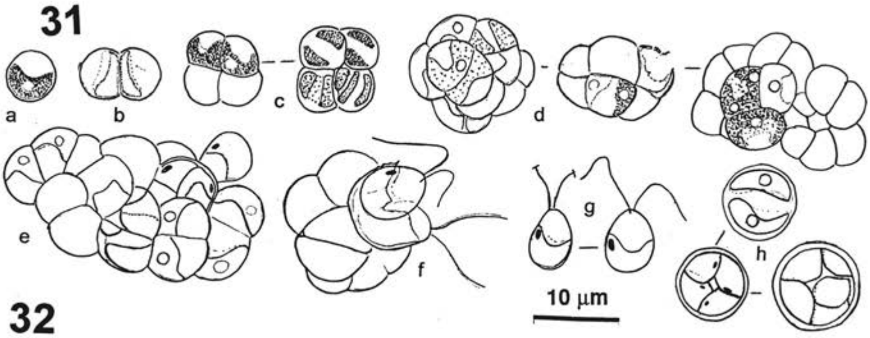

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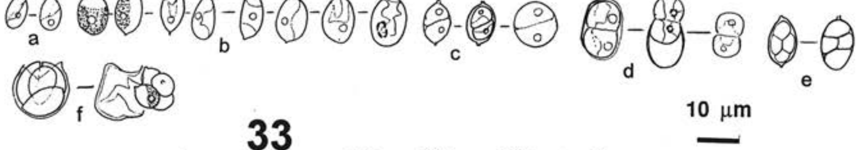

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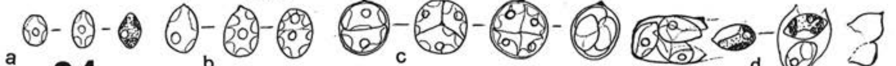

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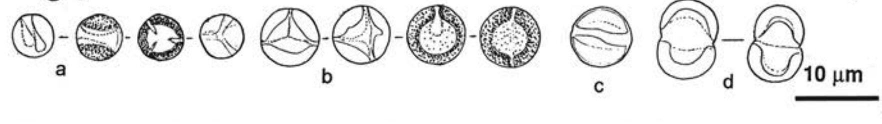

papillae at the poles; reproduction by (2-)4-8 autospores, 6-7.5 × 4-5 µm, produced with tetrahedral pattern.

This taxon was identified at the sites 13 and 16. The taxonomic position of this species is controversial, since it has unicellular stages similar to species of *Scenedesmus*. This species has been reported from moist soils of tropical regions, such as Cuba and Angola (Komárek & Fott, 1983).

* ***Oocystis asymmetrica*** **W. *et* G.S.West sensu Komáromy** (Fig. 33)

Cells solitary or in 2-4 celled colonies, oval to broadly oval, (5) 6-10 µm diam. or 6-10(12) × 5-8(10) µm, ends rounded with a papilla; chloroplast saucer-shaped, lobed, parietal, with pyrenoid, 1-2 per cell. Autosporangia common, 4-celled, 10-12 µm diam.

This taxon was identified at the site 13. Very common in irrigated places and on concrete walls near the ground (20 cm above soil surface). Komáromy (1975) described this species from soil near a river in the Matra Mountains (Hungary). Ettl & Gärtner (1995) mentioned it from soil in Australia. It has never been recorded from monuments. The epilithic forms growing in the field show mostly unicellular stages, but in liquid cultures this species forms sometimes berry-like colonies. The isolated strains show extensive morfological variability in BBM medium.

Muriella terrestris **J.B.Petersen** (Fig. 34)

This taxon was identified at the sites 3, 13 and 16. It is a typical soil alga, known also from monuments (Kapusta & Kovacik, 2000; Ortega-Calvo *et al.*, 1991, 1993).

Chlorella vulgaris **Beijerinck** (Fig. 35)

This taxon was identified at the sites 1, 3, 13 and 16. It forms monospecific green or yellowish green growths on subaerial substrates. This species is the most frequently cited green alga on monuments (Ortega-Calvo, 1993; Schlichting, 1961; Tomaselli *et al.*, 2000; Grant, 1982; John, 1988; Albertano *et al.*, 1991; Darienko & Hoffmann, 2003; Rindi & Guiry, 2003).

Chlorella **sp.** (Fig. 36)

Cells spherical, individual or in 2-4 celled groups, 4-8(10) µm diam.; young and old cells with vacuoles; chloroplast parietal, with small pyrenoid, often not clear; reproduction by autospores released by breaking of the mother cell wall.

◀

Figs 29-34. **29**. *Apatococcus lobatus*: *a* initial cells, *b* cell division, *c* vacuolized adult cell, *d, e* young colonies, *f, g* pseudofilaments. **30**. *Chlorosarcinopsis* sp.: *a* initial cells, *b* cell division, *c* four-celled colonies, *d* young colonies, *e* adult colony. **31**. *Chlorosarcinopsis* cf. *arenicola*: *a* initial cell, *b* cell division, *c* four-celled colonies, *d* young colonies, *e* adult colony, *f, h* zoosporangia, *g* zoospores. **32**. *Ecdysichlamys obliqua*: *a* initial cells with polar papilla, *b* matured cells, *c* two-celled autosporangia, *d* cell division, *e* four-celled autosporangia, *f* ruptured autosporangia. **33**. *Oocystis asimmetrica*: *a* young cells, *b* adult cells, *c* autosporogenesis, *d* autosporangia, *e* empty autosporangium. **34**. *Muriella terrestris*: *a* initial cells with one, two or three chloroplasts, *b, c* adult cells, *d* cell division. Top scale is applied to Figs 29-31; mid scale is applied to Fig. 32; bottom scale is applied to Figs 33-34.

This taxon was recorded at the site 16. This alga is morphologically close to *C. mirabilis* Andreyeva (Andreyeva, 1998), but could not be identified with certainty because of the small and unclear pyrenoid and the occurrence of vacuoles in all stages.

* ***Chlorella kessleri*** **Fott *et* Nováková** (Fig. 37)

Cells globular, solitary or in groups, 4-12 µm diam.; cell wall thin, chloroplast initially band-shaped or cup-shaped, subsequently covering more than 3/4 of the cell wall, with large pyrenoid, 2-4 µm diam. Reproduction by subglobular or elliptical autospores; autosporangia 12-17 µm diam.

This taxon was identified at the sites 2, 9, 13 and 16. It is known for subaerial habitats, mentioned from monuments (Kapusta & Kovacik, 2000). Similar and more common than this species is *C. reisiglii* (Reisigl) S. Watanabe, reported from monuments (Andreyeva, 1998; Leitao *et al.*, 1996; Ortega-Calvo *et al.*, 1991, 1993).

Scenedesmus obtusiusculus **Chodat** (Fig. 38)

This taxon was identified at the site 16. Known from small water-slops, from clean water reservoirs and from soil in Japan, Switzerland and Hungary (Komárek & Fott, 1983). This species shows a strong variability in the shape of cells and number of cells of cenobia. It is similar to *S. obliquus* (Turpin) Kütz., but coenobia of *S. obliquus* have cells straight and bigger (22 × 8 µm) and the alternation is looser (Hindák, 1990).

CHAROPHYTA

* ***Klebsormidium nitens*** **(Menegh. in Kütz.) Lokhorst** (Fig. 39)

In liquid medium (BBM) extensively forming delicate, free-floating, submerged tufts, composed of short and straight or slighty twisted filaments. In agar medium (BBM) forming a homogeneous layer, composed of short (max 70-celled) and straight or slightly bent filaments, dark-green. Apical cell obtuse, intercalary cells cylindrical. Cell dimensions (5.8)6-6.5(7) µm in width by 5.8-12(16) µm in length. Chloroplast in cells typically parietal, plate-shaped, with one pyrenoid, remarkably elongated. Vegetative reproduction by fragmentation of filaments. Asexual reproduction by formation of biflagellate zoospores, formed singly in an individual cell or in a series of adjacent cells, 8-10 × 5-6.5 µm, pyriform, dorsiven-

▶

Figs 35-39. **35**. *Chlorella vulgaris*: *a* initial subspherical cells, *b* young cells, *c* adult cells, *d* cell division, *e* old cells, *f* autosporangia, *g* ruptured autosporangia, *h* empty autosporangium. **36**. *Chlorella* sp.: *a* young cells, *b* adult cells with one vacuole, *c* autosporangium, *d* ruptured autosporangium. **37**. *Chlorella kessleri*: *a* young cells, *b* adult cells, *c* cell division, *d* two planes division, *e* old vacuolised cell, *f* ruptured autosporangium. **38**. *Scenedesmus obtusiusculus*: *a* young cells, *b* cells with two chloroplasts, *c, d* autosporangia, *e-h* cenobia, *i* ruptured autosporangia. **39**. *Klebsormidium nitens*: *a-h, m, n* fragments of filaments, *i-l* filaments, *o* zoospores, *p* initial stadium with polar mucilaginous attachment. Top scale is applied to Figs 35-37; bottom scale is applied to Figs 38-39.

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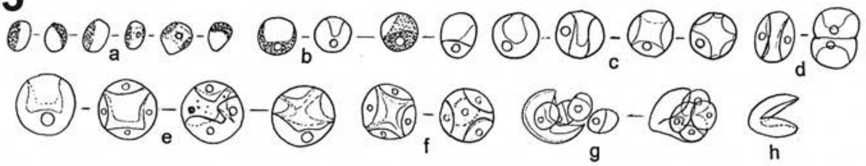
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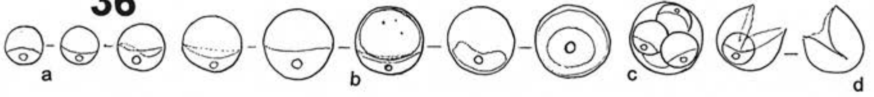
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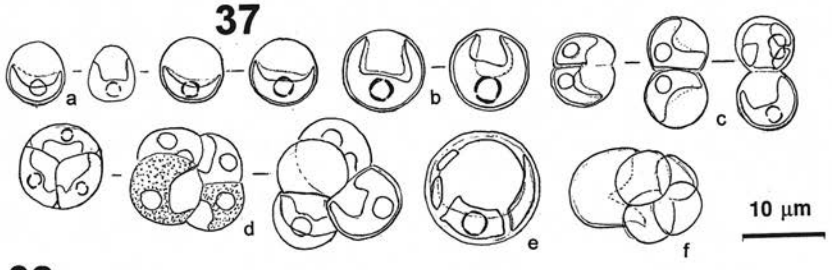
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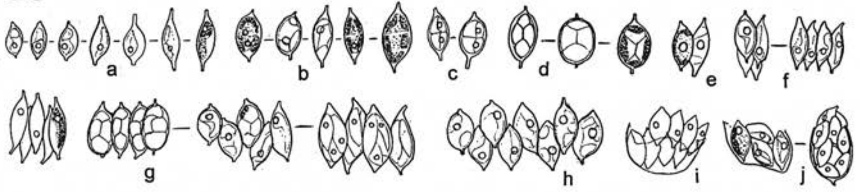
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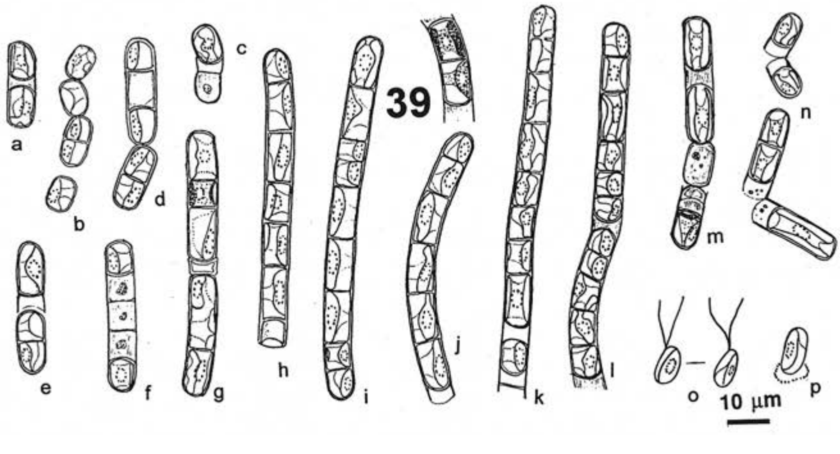
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tral, with a plate-like chloroplast provided with a small but distinct pyrenoid. The aperture of escape of the zoospores forms a well-visible pore in emptied cells.

This taxon was identified at the sites 14 and 16. This alga has a widespread distribution in Western and Central Europe. Lokhorst (1996) studied this species from collections in the Netherlands, made on piles and stones at the water surface in an alkaline lake. Records of this species are available for Belgium (collected from a sheet-piling of the Schelde-Maas kanaal near Lommel, in swamps), France (from an algal mat in the dripping-zone of a rock near Plombieres les Bains), Switzerland (from samples scraped from sheet-piling and stones bordering the Wallen See near Wallenstaft) and Austria (collected from stones in the river Leutascher Ache near Leutasch, from soil samples from the Karwendel Mountains and from the bank of the stream Finsterbach near the foot of the Hohe Münde) (Lokhorst, 1996). This species has not been reported as biodeteriogen from monuments so far.

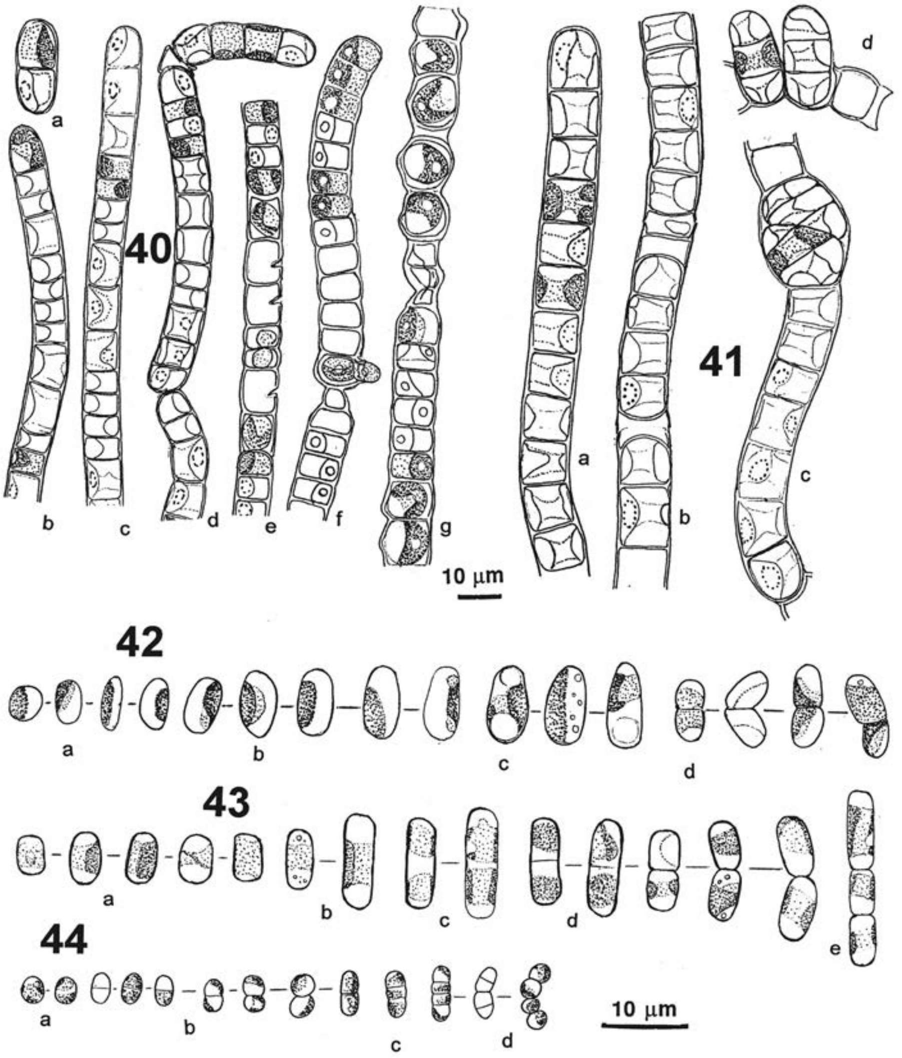
***Klebsormidium flaccidum* (Kütz.) Silva, Mattox *et* Blackwell** (Fig. 40)

This taxon was identified at the sites 9 and 13. This species appears to be widely distributed in Western Europe. Lokhorst cultured this species from material collected in Germany (from a soil sample removed from clay soil in a field of beets near Niedrekrüchten), in the Netherlands (an algal coat extensively covering the base of a stem of *Quercus* near Dwarsgracht), in Belgium (from a soil sample taken from the slope of a brooklet near Baarle-Nassau), in France (from a fountain near Vittel and a soil sample from a forest path near St. Céré). This species is very well known as biodeteriogen of monuments (Schlichting, 1975; Ortega-Calvo *et al.*, 1991, 1993; Leitao *et al.*, 1996; Wasser *et al.*, 1998; Kapusta & Kovacik, 2000; Darienko & Hoffmann, 2003). Culture is usually needed for correct determination of species of *Klebsormidium*. The majority of records of *Klebsormidium* in terrestrial habitats are referred to *K. flaccidum* (John, 1988). However, in several studies no culture was used and it is therefore possible that some records of *K. flaccidum* are incorrect.

* ***Klebsormidium crenulatum* (Kütz.) Lokhorst** (Fig. 41)

Limited growth of filaments observed in liquid medium, resulting in relatively small, dense, tufts of indefinite shape, mostly 0.5-1 cm wide, bright green, composed of intimately and spirally twisted filaments (50-85 cells); production of zoospores not observed. Profuse growth observed on agar, forming a green compact layer exhibiting rough to wrinkled surface. Old agar cultures gradually becoming a wavy accumulation, producing an amorphous algal mass with filaments becoming bent and twisted. Apical cells obtuse, especially in germinating stages originating from zoospores or aplanospores. Filaments consisting of uniseriate cell rows, which are randomly interrupted by cell doublets produced by transversal septation of the filament. In case of production of aplanospores, the germinating aplanospores may give rise to a pseudobiseriate configuration. In germinating stages, cells are 9.5-10 (11) µm diam., wider than long. In well-developed filaments, the cells are 11-13(13.8) µm wide. Chloroplast parietal, plate- to girdle-shaped, with one pyrenoid.

This taxon was identified at the site 16. Lokhorst studied this alga from material collected in Slovakia (from a burned pine forest near Malacky), in Austria (from a soil sample taken from the eastern slope of the mountain Hohe Münde near Leutasch), in the Netherlands (from a soil sample collected in the vicinity of acid and oligotrophic ehemeral pools in loam-pits near Vierhouten), in



Figs 40-44. **40**. *Klebsormidium flaccidum*: *a* initial stadium, *b, c* vegetative filaments, *d* fragmentation, *e, f* empty zoosporangia, *g* autosporangia. **41**. *K. crenulatum*: *a, b* filaments, *c* filament with autosporangium, *d* young growing filaments. **42**. *Stichococcus allas*: *a* initial cells, *b* adult cells, *c* vacuolized old cells, *d* cell division. **43**. *Stichococcus bacillaris*: *a* initial cells, *b* elongated cells, *c* cells with two chloroplasts, *d* cell division, *e* pseudofilaments. **44**. *Stichococcus minutus*: *a* initial cells, *b* cell division, *c, d* three- and four-celled filaments. Top scale is applied to Figs 40-41; bottom scale is applied to Figs 42-44.

Belgium (from a soil sample taken from the clayey slope of the river Mark near Meerle) and in France (from an algal mass growing near the water surface on aquatic plants in a stream near the Lac des Settons). *K. crenulatum* is mentioned by Uher & Kovacik (2002b) from an historical cemetery in Bratislava (Slovakia) and by Franzen *et al.* (2002) as an aggressive colonizator of industrial areas of the Region of Nordheim-Westfahlen (Germany). This alga appears to have a widespread distribution in Europe.

* *Stichococcus allas* Reisigl (Fig. 42)

Cells mainly solitary, elliptical, obovate or gutter-shaped, vacuolized, 5-8 × 3-6 µm, with a band-shaped chloroplast.

This taxon was identified at the site 13. Similar to this species is *S. chlorelloides* Grintzesco & Péterfi. *S. allas* has not been recorded from monuments.

Stichococcus bacillaris Nägeli (Fig. 43)

This taxon was identified at the sites 1, 9, 13, 14 and 16. It forms pseudofilaments in cultures. It is a very common terrestrial species, known from buildings of Rome in Italy (Grilli Caiola *et al.*, 1987), Baton Rouge and Houston in USA (Brook, 1968), Coimbra in Portugal (Leitao *et al.*, 1996), Sevilla and other cities of Spain and Italy (Ortega-Calvo *et al.*, 1991, 1993), Michigan and Texas, USA (Schlichting, 1961, 1969), Bratislava in Slovakia (Kapusta & Kovacik, 2000), Moscow in Russia (Wasser *et al.*, 1988), Galway in Ireland (Rindi & Guiry, 2003), Kyiv in Ukraine (Darienko & Hoffmann, 2003) and Venice in Italy (Andreoli & Rascio, 1982).

Stichococcus minutus Grintzesco *et* Péterfi (Fig. 44)

This taxon was identified at the site 16. It is known as biodeteriogen of monuments from Bratislava (Kapusta & Kovacik, 2000) and from SE Spain (Aboal, 1996). Other species of *Stichococcus* recorded from monuments include *S. chlorelloides* Grintzesco & Péterfi in Russia (Wasser *et al.*, 1988).

General considerations

With regard to the number of epilithic and endolithic species found, the sites 16, 13 and 9 supported the highest diversity of species among all sites investigated (respectively 24, 17 and 13). Whereas at the sites 16 and 13 these were mainly coccoid green algae, at the site 9 they were predominantly coccoid blue-green algae (8 species). The conditions of the habitats sampled had a major influence on the species composition; the site 4 was colonized only by one endolithic species, *Chroococcidiopsis kashaii*, and the site 10 was colonized by two species, *Synechocystis* sp. 2 and *C. kashaii*. Monuments characterized by a high variability of habitats were colonized by many species (for example the site 9). The sites 14 and 15, which represented a special type of habitat characterized by harsh conditions (chalk springs) showed a low diversity (3 taxa). Some species were widespread and occupied a large range of habitats; these included some of the most frequent taxa, such as C*hroococcidiopsis kashaii*, *Nostoc sphaericum*, *Botrydiopsis* sp., *Apatococcus lobatus*, *Chlorosarcinopsis* cf. *arenicola*, *Muriella terrestris, Chlorella vulgaris, Chlorella kessleri* and *Stichococcus bacillaris. Leptolyngbya* was the richest genus (7 taxa).

To date, studies concerning the distribution of cyanobacteria and microalgae on monuments are entirely descriptive. Previous studies on the European subaerial phycoflora have considered the composition of subaerial algal assemblages to be affected by environmental factors operating on both small, local scales (i.e., moisture, temperature, light irradiation, substratum) and large, macroclimatic scales (i.e., climatic zones). The first comprehensive studies focused on subaerial algal vegetation in Europe are those by Kalchbrenner (1866), Scherfel (1902, 1907), Novácek (1934), Jaag (1945) and Golubić (1967). These works concerned mostly calcareous or alkaline natural substrata and their results generally showed the predominance of cyanoprokaryotic elements in the subaerial vegetation. Similar conclusions were obtained in more recent studies, such as Johansen *et al.* (1983), Hoffmann (1989), Chang (1994), Pantazidou (1996), Büdel (1996) and Uher & Kovacik (2002a). Epilithic and endolithic species commonly occur on most subaerial stone surfaces and colonize artificial substrata more or less quickly, depending on environmental factors such as light irradiation, water retention and availability of organic and inorganic compounds (Kovacik, 2000). The phycoflora of monuments has been studied in Spain (Ortega-Calvo *et al.*, 1991, 1993; Rifón-Lastra & Noguerol-Seoane, 2001), Portugal (Leitao *et al.*, 1996), Greece (Anagnostidis *et al.*, 1992), Italy (Tomaselli *et al.*, 1982; Andreoli & Rascio, 1987; Lamenti *et al.*, 2000; Tomaselli *et al.*, 2000), Slovakia (Kapusta & Kovacik, 2000; Uher & Kovacik, 2002b; Godyová *et al.*, 2003; Uher & Godyová, 2003), Russia (Wasser *et al.*, 1988), Ukraine (Wasser *et al.*, 1988; Darienko & Hoffmann, 2003), USA (Schlichting, 1961; Brook, 1968), Great Britain (Ortega-Calvo *et al.*, 1993), Ireland (Schlichting, 1975; Rindi & Guiry, 2003) and Canada (Brook, 1968). Our investigation showed that the algal growths of more humid sampling sites were dominated by green algae. In our case, coccoid green algae were the dominant forms at these sites; this is in contrast with the situation reported for Atlantic parts of Europe, where filamentous forms, such as species of *Trentepohlia* and Prasiolales are often the most common algae (e.g. John, 1988; Noguerol-Seoane & Rifón-Lastra, 1997; Rindi *et al.*, 1999; Rindi & Guiry, 2002). On the other hand, cyanobacteria dominated in semiarid conditions.

Calcareous rock (limestone) is the main building material used for monuments in Murcia. Our investigation showed that the monuments investigated were mainly colonized by endolithic or chasmoendolithic cyanobacteria, which require low level of illumination and minimal fluctuations of seasonal temperature. Species of *Chroococcidiopsis* are important biodeteriogens of many buildings (Ortega-Calvo & Ariño, 1994); although occasionally mentioned for monuments (Ortega-Calvo *et al.*, 1993), they have been most frequently reported for caves and underground sites (Palik, 1938; Friedmann, 1961; Claus, 1962; Komáromy, 1975; Abdelahad, 1985; Anagnostidis & Komárek, 1985; Asencio & Aboal, 2000). Similar observations are available in the literature for limestone surfaces in similar climatic conditions, although marble is the type of artificial rocky substratum that has been mostly investigated in urban areas. Information on marble surfaces is available for monuments in Italy (Giaccone *et al.* 1976; Danin & Caneva, 1990), Greece and Turkey (Anagnostidis *et al.*, 1992); since marble has been largely used for monuments in these countries, such investigations were mostly applied studies, which focused on the process of colonization and destruction of marble by cyanobacteria. The two sites characterized by marble surfaces in our study showed a low diversity, as samples collected from this substratum supported no more than 2 species. High evaporation rates or low levels of nutrient supply may affect negatively species diversity on marble surfaces (Darienko & Hoffmann, 2003), but further investigations are necessary to clarify the processes responsible of this phenomenon.

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