Short Note

Evaluation of methods for retrieving foraminifera from indurated carbonates: application to the Jurassic spongiolithic limestone lithofacies of the Prebetic Zone (South Spain)

Matías Reolid[1*] and Concha Herrero[2]

[1*]*Departamento de Estratigrafía y Paleontología, Facultad de Ciencias, Universidad de Granada, Fuentenueva s/n, 18071 Granada (Spain)*

[2]*Departamento de Paleontología, Facultad de Ciencias Geológicas, Universidad Complutense, 28040 Madrid (Spain).*

email: mreolid@ugr.es, cherrero@geo.ucm.es

ABSTRACT: Two methods for retrieving foraminifera from strongly lithified carbonates (Amine-O and cold-disaggregation with acetic acid) were tested on the same sample of Oxfordian spongiolithic limestone (Prebetic Zone, SE Spain) and compared with thin section analysis. Differences between the methods concern: 1) weight of sieved residues after disaggregation; 2) foraminifera/gram ratio; 3) preservation features of the tests; 4) foraminiferal assemblage compositions. The results obtained allow us to conclude that accurate paleoecological and taxonomical analysis of indurated carbonates requires the combined use of thin sections and disaggegration treatment of the samples.

INTRODUCTION

In many basins, recovering foraminifera from indurated limestones poses considerable problems. Traditional techniques such as simple water soaking or methods using hydrogen peroxide (H_2O_2) or washing-soda (Na_2CO_3) are useful in disaggregating marls, marly limestones and partially indurated argillaceous limestones, but are ineffective in extracting calcareous microfossils from strongly lithified carbonate matrix rocks. Oxfordian foraminiferal assemblages largely consist of various agglutinated, porcelaneous and hyaline taxa that require the study of external morphology for accurate genus and species identification, and so clean, isolated specimens are necessary for proper taxonomical and paleoecological studies. Techniques involving surfactants, such as the no longer available Quaternary-O (Zingula 1968), Miramine OC-ES (Thomas and Murney 1985) and Amine-O (Ruget et al. 1989, Bennington 1993), or reactives like hydrofluoric acid (Magné and Dufaure 1964), hydrochloric acid (Moura et al. 1999) and acetic acid (Bourdon 1962, Thomas and Murney 1985, Lethiers and Crasquin-Soleau 1988, Moura et al. 1999, Lirer 2000) have been successfully employed in different ways to extract calcareous microfossils from mudstones, indurated sandstones, shales and hard argillaceous limestones. Time required for disaggregation using surfactants and acids has been quite well specified, and varies from less than an hour (Magné and Dufaure or Ruget et al.) to several hours (Bennington, Bourdon, Lirer, Moura et al. or Zingula), or even several days or weeks (Thomas and Murney or Lethiers and Crasquin-Soleau). Most of the above mentioned treatments indicate that undamaged shells are recovered except for corroded foraminiferal shells in Magné and Dufaure's hydrofluoric acid treatment; however very little information is given showing how the reagents affect specimen preservation and assemblage composition. The aim of this study is to test the effectiveness of two such methods (Amine-O and acetic acid) and to compare the results in terms of number of recovered specimens, test preservation and assemblage composition.

MATERIAL

The Oxfordian rocks in the Prebetic Zone of the Betic Cordillera (SE Spain) mostly comprise indurated nodular-like limestones and marly calcareous rhythmites at some intervals, interpreted as pelagic-hemipelagic carbonate sedimentation in an epicontinental shelf system (Olóriz et al. 2002). The sample used in this study was obtained from level 3 of the El Chorro section (CHO) located in the External Prebetic. According to the ammonite assemblages, the level studied is Upper Oxfordian in age, the Bifurcatus Zone of the biochronostratigraphical zonal scheme of Olóriz et al. (1999). The CHO-3 level is included in the spongiolithic limestone lithofacies of Olóriz et al. (2002), and is a well-stratified reddish limestone bed, 25-30cm thick, characterized by a high abundance of dictyid siliceous sponges, which constitute the main component of this lithofacies.

DISAGGREGATION METHODS AND LABORATORY ROUTINES

A slight modification of the Amine-O method proposed by Ruget et al. (1989), and steps 1 to 4 of the cold-disaggregation with acetic acid treatment of Lirer (2000, p. 366, text-fig. 3) were applied to two sub-samples of the same Upper Oxfordian spongiolithic limestone sample collected in CHO.

Amine Oxide, usually called Amine-O, is a non-ionic industrial surfactant liquid that for this micropaleontological technique must be combined with acetic acid and water in the following

Present address: Department of Geology, University of Oslo, P. O Box 1047, Blindern, N-0316 Oslo, (Norway). E-mail: m.r.prez@geo.uio.no

proportions: 40ml Amine-O, 4ml pure acetic acid (99%) and 456ml warm distilled water; the solution is then allowed to rest for 24 hours. The processing steps are: a) break 300g of the sample into fragments of about 5mm in diameter and oven-dry them at 40-50ºC; b) place the crushed sample in a saucepan and cover it with the prepared Amine-O; c) heat and stir on an electric hotplate until the solution comes to a boil; d) reduce the temperature of the hotplate and continue applying heat for 10 minutes with constant stirring; e) add 225ml hydrogen peroxide 30% and immediately wash the sample.

The treatment in cold-disaggregation with acetic acid is as follows: a) break 300g of the sample into fragments of about 5mm in diameter; b) place the crushed sample in a glass beaker and cover it with a solution of acetic acid made up of 80% CH_3COOH and 20% distilled water (the level of acid must be at least 2cm higher than the sample level); c) keep the sample submerged in the solution for 10 hours in a fume cupboard; d) finally, wash the sample with abundant water.

The washing procedure was the same in both methods. The disaggregated sub-samples were washed through a column of standard stainless steel sieves with mesh openings of 1000, 500, 250, 125 and 60μm, with a gentle jet of water from the tap. The residues were oven-dried at low temperature (40-50ºC) and transferred to labeled paper bags. The foraminifera were hand-picked with a hair paint-brush and distilled water on a standard black picking grid-tray under stereoscopic microscope (Wild M-10 and Leica MZ-12).

The specimens illustrated in the plate were mounted on an aluminum stub, gold-coated and photographed by scanning electron microscope (SEM, Jeol-JSM-6400). All the material studied and illustrated is deposited in the Departamento de Estratigrafía y Paleontología of the Facultad de Ciencias of the Universidad de Granada (Spain). The taxa are arranged in accordance with the classification proposed by Loeblich and Tappan (1988).

RESULTS

Thin section analysis

Microfacies analysis of the CHO-3 level was performed on 3 standard thin sections (Reolid 2003). The fabric is normally grain-supported, and locally matrix-supported. The depositional texture shows a transition between packstone and wackestone. The grains are mainly microbial oncoids with nubeculariids (33.9%) and bioclasts (22.3%) such as fragments of echinoderms, foraminifera and sponge spicules. The rest of the grains are lumps, ooids, tuberoids, peloids, aggregate grains and scarce iron oxides.

On analysing two thin sections from the selected level, 800 specimens of foraminifera (excluding sessile taxa, e.g. nubeculariids) were counted (Reolid 2003). The assemblage is dominated by planktic specimens of the genus *Globuligerina* Bignot and Guyader 1971 (55.4%), the second most significant component being free-living benthic foraminifera (42.1%); the sessile foraminifera chiefly belong to nubeculariids and to some siliceous agglutinated foraminifera. Among free-living benthic taxa, the suborders Textulariina (47.3%), Spirillinina (27.8%) and Lagenina (18.3%) are the principal components; other free-living benthic foraminifera such as Robertinina are scarce. The main limitation encountered in this approach is the difficulty in distinguishing the species and some genera, as is the case of Lagenina (e.g. *Nodosaria* Lamarck 1812 and *Dentalina* Risso 1826, *Lenticulina* Lamarck 1804 and *Astacolus* de Montfort 1808) and nubeculariids (e.g. *Nubecularia* Defrance 1825 and *Nubeculinella* Cushman 1930).

Amine-O Method

The 300g processed sub-sample was reduced to 279.51g. The weight residues in the different sieves are given in text-figure 1-A. The complete or partial picking of the fractions yielded a total of 1002 specimens. The foraminifera/gram ratio increases towards the finer fraction and varies from 1.03 in the 1000-500μm sieve to 1268.19 in the 125-60μm one.

Visual and binocular analysis of the residues and specimens revealed no color change between the rock and the processed sub-sample. In all the fractions, the foraminifera retrieved were usually dirty, the test surfaces being covered with clay-sized carbonate particles. This fact impeded recognition of the specimens and obscured important features of shell morphology. Sometimes taxonomical identification was almost impossible; indeterminate specimens are abundant in the >125μm fractions (text-fig. 1-C). Test surfaces in the lamellar radiate hyaline group (Lagenina and Globigerinina), the porcelaneous group (Miliolina) and the single crystal hyaline group (Spirillinina, *Spirillina* Ehrenberg 1843) seem to be unaffected by corrosion arising from the treatment (Pl. 1, figs 1-4).

The assemblage composition is dominated by free-living benthic taxa (text-figs 1-B and C). Spiral and uniserial specimens of Lagenina and uniserial agglutinated forms are the main components in the coarser fractions. A sharp decrease in Textulariina and an increase in Spirillinina and Miliolina (ophthalmidiids) occur in the two finest fractions (<250μm); the spiral forms decrease compared to uniserial morphologies in Lagenina. The planktic specimens belonging to the genus *Globuligerina* are mainly found in the fractions <250μm.

Acetic acid Method

The acetic acid method reduced 300.1g of the spongiolithic limestone sub-sample to 237.99g of total residue, with the >1000μm fraction accounting for 210.09g (see text-fig. 1-A). A total of 3680 specimens were retrieved in the complete or partial picking of the sieved fractions. The foraminifera/gram ratio shows an increase from the coarser fractions to the fine-grained ones.

The first feature to draw our attention was the color loss on the grains and on the test surfaces with respect to the original reddish color of the rock sample. It is also surprising how some foraminifera preserved as internal molds, e.g. *Lingulonodosaria* Silvestri 1903, were destroyed during the picking simply by contact with the distilled water and the hair paint-brush. Although clay-sized carbonate particles were observed on the test surfaces, recognition and identification of the taxa was usually possible; the proportion of indeterminate foraminifera being low (text-fig. 1-C). SEM inspection of test surfaces showed a slight degree of corrosion due to the sample treatment (pl. 1, figs 5-7); some *Lagenina* specimens also presented minor enlarged and coalescing pores (pl. 1, fig. 8) due to corrosion and dissolution.

The foraminiferal assemblage was dominated by free-living benthic taxa (text-figs 1-B and C). Agglutinated and hyaline planispiral Lagenina taxa were the main components in the 1000-500μm and 500-250μm fractions. A strong increase in

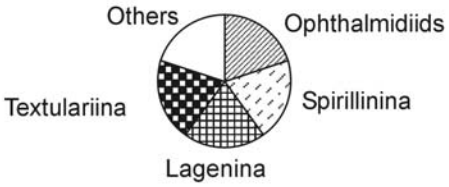
A

Amine-O

Grain size	Weight (g)	foram/g
> 1000 µm	248.32	—
1000-500 µm	13.53	1.03
500-250 µm	7.78	7.06
250-125 µm	5.72	246.00
125-60 µm	4.16	1268.19

Acetic acid

Grain size	Weight (g)	foram/g
> 1000 µm	210.09	—
1000-500 µm	10.32	3.19
500-250 µm	6.66	34.82
250-125 µm	7.09	663.91
125-60 µm	3.83	6797.81



B

Amine-O

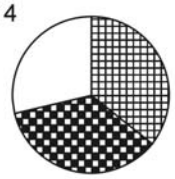
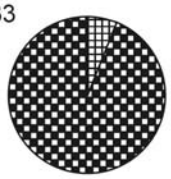
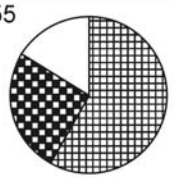
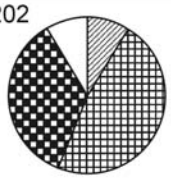
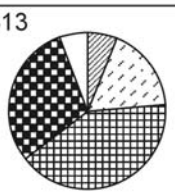
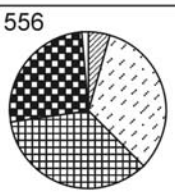
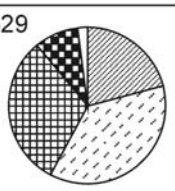
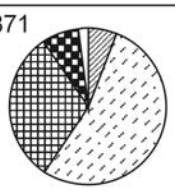
Grain size	Planktic	Benthic	Ophthalmidiids	Spirillinina	Lagenina	Textulariina	Others
1000-500 µm	0.00	100.00	0.00	0.00	35.71	35.71	28.58
500-250 µm	0.00	100.00	0.00	0.00	58.18	25.46	16.36
250-125 µm	3.12	96.88	6.08	17.88	40.58	30.03	5.43
125-60 µm	13.28	86.72	20.80	37.05	31.01	9.26	1.88

Acetic acid

Grain size	Planktic	Benthic	Ophthalmidiids	Spirillinina	Lagenina	Textulariina	Others
1000-500 µm	0.00	100.00	0.00	0.00	6.06	93.94	0.00
500-250 µm	12.93	87.07	8.91	0.00	47.03	35.64	8.42
250-125 µm	28.33	71.67	4.56	32.97	35.34	26.12	1.01
125-60 µm	29.84	70.16	5.70	53.48	31.01	7.98	1.83

C

Benthic assemblages according to the processing method

Grain size	Amine-O	Acetic acid
1000-500 µm		
500-250 µm		
250-125 µm		
125-60 µm		

TEXT-FIGURE 1
Sub-sample residues, foraminifera /gram ratio and foraminiferal assemblage compositions in the Amine-O and acetic acid methods. A: Weight residues for each fraction and foraminifera per gram in the two methods. B and C: Assemblage composition for each fraction in the two methods; "Others" includes indeterminate specimens and accessory taxa (ceratobuliminids and nubeculariids).

Spirillinina was observed in the <250µm fractions; *Textulariina* decreased and, within Lagenina, uniserial forms increased and planispiral morphologies decreased. *Globuligerina* was identified in the 500-250µm residue and increased by up to 29.84% in the 125-60µm fraction (text-fig. 1-B).

DISCUSSION AND CONCLUSIONS

The different features found in the specimens and assemblage compositions as a result of the two disaggregation techniques must be inherent to the treatment, as the two sub-samples were obtained from the same sample.

With regard to sample preparation, cold disaggregation with acetic acid is more effective than the Amine-O method because the acid-treated sub-sample is better disaggregated and more clay particles are eliminated. This fact produces a lower quantity of washed residues and therefore a greater concentration of foraminifera per gram.

Concerning the preservational features, cleaner specimens were obtained with the acetic acid technique, which enabled better recognition of foraminifera; this could also explain the higher proportion of foraminifera per gram resulting from the acetic acid treatment. However, this method generates slight signs of corrosion on shell surfaces and probably the loss of foraminifera preserved as internal molds.

The two methods produced only slight differences in assemblage compositions except for those related to the 1000-500m and 500-250m sieves, the ophthalmidiids and the abundance of indeterminate specimens. The number of specimens in the coarser fractions is low and so it is not statistically representative; this could explain the variations found between the two methods in the 1000-500m and 500-250m sieves. Concerning the test composition in Miliolina (HMC), the ophthalmidiid shells could be especially sensitive to dissolution effects under the acetic acid attack, which could explain the higher proportion of *Ophthalmidium* Kübler and Zwingli 1870 found using the Amine-O technique. Finally, the higher values of indeterminate specimens found with the Amine-O method mainly arose from the impossibility of taxa identification due to the presence of more clay-sized carbonate particles attached to the test surfaces than with the acetic acid method.

Comparison of the results of the thin section and sieved residue analysis reveals an important difference in the percentage of sessile and planktic foraminifera. In thin section, nubeculariids and other attached forms are significant qualitative components in the growth of microbial oncoids, which are one of the main components of the microfacies. However, they cannot be readily identified in the sieved residues either with the Amine-O technique or with the acetic acid method. The dissimilarity found in the number of specimens of *Globuligerina* could be due to the difficulty in recognizing them in released specimens, where their small size and the particles attached to their test surface obscure the sutures and the morphology of the chambers.

From the above comparison of the Amine-O and cold-disaggregation with acetic acid methods, in conjunction with the thin section analysis, we conclude that accurate taxonomical and paleoecological studies in strongly lithified limestones require the combined use of released specimens and sectioned rock sample.

ACKNOWLEDGMENTS

We greatly acknowledge the useful comments of Frank C. Thomas and Jenø Nagy, who undertook the pre-review of our manuscript. The SEM photographs were taken at the Centro de Microscopía Electrónica "Luis Brú" of the Universidad Complutense of Madrid. This work was supported by the projects BTE2001-1924 and BTE2001-3029, the EMMI group (RNM-178, Junta de Andalucía) and a special fellowship (Matías Reolid) from the Universidad de Granada to visit the Departamento de Paleontología (Universidad Complutense, Madrid).

REFERENCES

BENNINGTON, J.B., 1993. Technique for disaggregating mudstones to retrieve microfossils and non-clay residual particles. *Journal of Paleontology,* 67: 154-155.

BOURDON, M., 1962. Méthode de dégagement des microfossiles par acétolyse à chaud. *Compte Rendu sommaire de la Société géologique de France,* p. 267-268.

LETHIERS, F. and CRASQUIN-SOLEAU, S., 1988. Comment extraire les microfossiles à tests calcitiques des roches calcaires dures. *Revue de Micropaléontologie,* 31: 56-61.

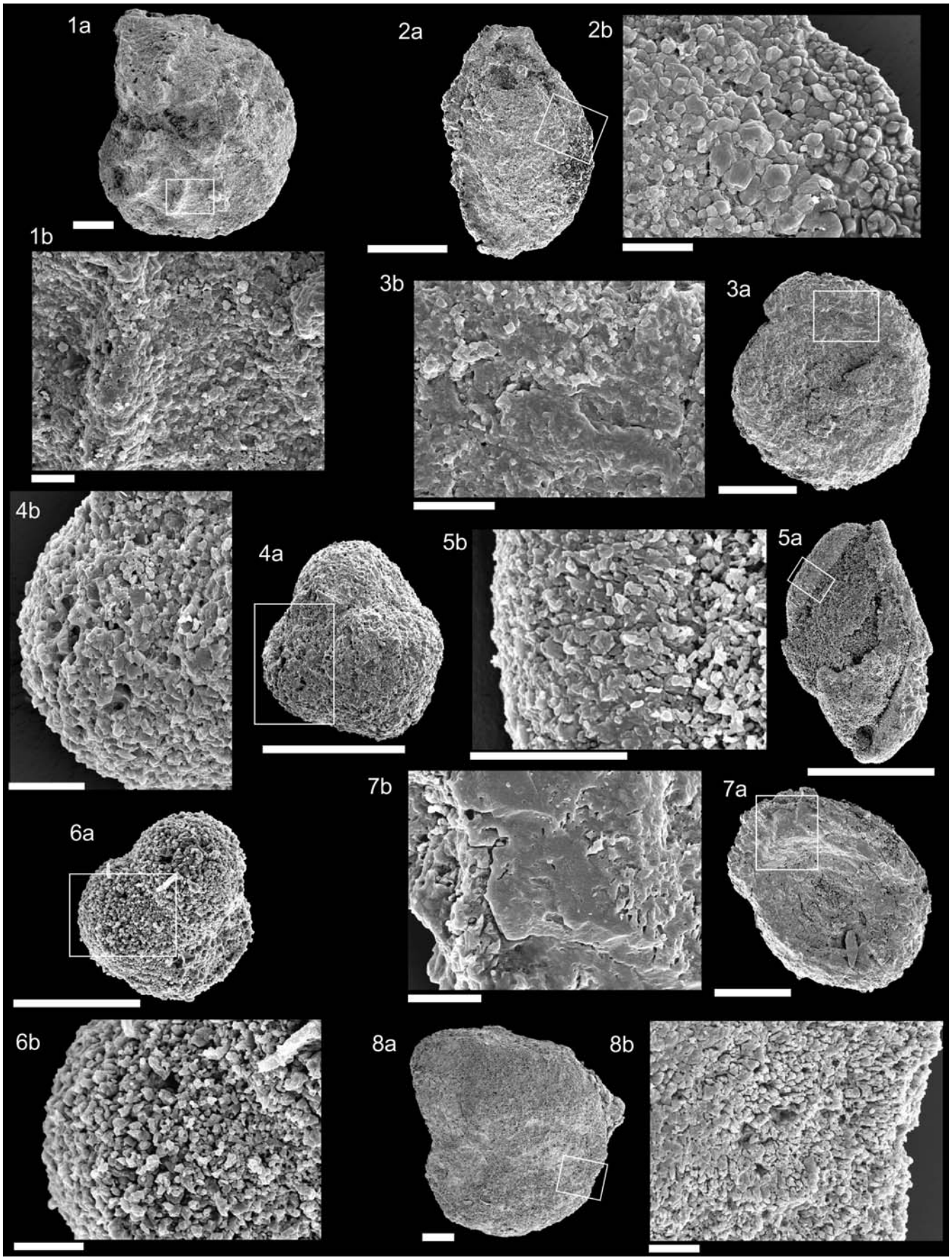
LIRER, F., 2000. A new technique for retrieving calcareous microfossils from lithified lime deposits. *Micropaleontology*, 46: 365-369.

PLATE 1

Scale bars in the general view of the specimens (figs 1a-8a) are 100µm; scale bars in detailed photographs (figs 1b-8b) are 20µm.

1-4 Examples of *Lenticulina*, *Ophthalmidium*, *Spirillina* and *Globuligerina* specimens recovered with the Amine-O method. The shell surface details show specimens undamaged by the treatment except for the slight corrosion visible in *Globuligerina* (specimen 4a-b).

5-8 Examples of *Ophthalmidium*, *Globuligerina*, *Spirillina* and *Lenticulina* specimens retrieved with the acetic acid method. Slight corrosion of shell surfaces is visible in details 5b, 6b and 7b. The *Lenticulina* specimen (fig. 8a,b) shows more significant corrosion and dissolution of the shell surface where enlarged and coalescing pores are visible.


1a
2a
2b
1b
3b
3a
4b
4a
5b
5a
7b
7a
6a
6b
8a
8b

LOEBLICH, A.R.Jr. and TAPPAN, H., 1988. *Foraminiferal genera and their classification.* New York: Van Nostrand Reinhold Company, 2 vol., 970pp.

MAGNÉ, J. and DUFAURE, G., 1964. Une méthode nouvelle pour l'extraction rapide des microfossiles. *Revue de Micropaléontologie,* 7: 77-79.

MOURA, J.C., RIOS-NETTO, A.M., WANDERLEY, M.D. and SOUSA, F.P., 1999. Using acids to extract calcareous microfossils from carbonate rocks. *Micropaleontology*, 45: 429-436.

OLÓRIZ, F., REOLID, M. and RODRÍGUEZ-TOVAR, F.J., 1999. Fine-resolution ammonite biostratigraphy at the Río Gazas-Chorro II section in Sierra de Cazorla (Prebetic Zone, Jaén province, southern Spain). *Profil*, 16: 83-94.

———, 2002. Fossil assemblages, lithofacies, taphofacies and interpreting depositional dynamic in the epicontinental Oxfordian of the Prebetic Zone, Betic Cordillera, southern Spain. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 185: 53-75.

REOLID, M., 2003. *Dinámica eco-sedimentaria durante el Oxfordiense medio-Kimmeridgiense temprano en la Zona Prebética: interpretación ecoestratigráfica y secuencial.* Unpublished Ph.D. Thesis, Universidad de Granada, 254 pp.

RUGET, C., CUBAYNES, R., NICOLLIN, J.P. and ROQUET, A.M., 1989. Une méthode de traitement (prélèvement, lavage, tri) pour l'analyse paléoécologique appliquée aux nodosariidés du Toarcien des coupes de Penne et de Caylus (Quercy, France). *Revue de Micropaléontologie,* 32: 45-52.

THOMAS, F.C. and MURNEY, M.G., 1985. Techniques for extraction of foraminifers and ostracodes from sediment samples. *Canadian Technical Report of Hydrography and Ocean Sciences,* 54: vi + 24 pp.

ZINGULA, R.P., 1968. A new breakthrough in sample washing. *Journal of Paleontology,* 42: 1092.

Manuscript received July 3, 2004
Manuscript accepted July 14, 2004