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An acid-free method of microfossil extraction from clay-rich lithologies using the surfactant Rewoquat

Emilia Jarochowska, Petra Tonarová, Axel Munnecke, Lenka Ferrová, Jan Sklenář, and Stanislava Vodrážková

ABSTRACT

Marine rocks characterized by high clay content provide excellent conditions for fossil preservation, particularly for organic-walled microfossils such as retiolitid graptolites and chitinozoans. Nevertheless, the phyllosilicate minerals, which constitute the clay component, make microfossil extractions difficult. The problem results from the tendency of phyllosilicates to form aggregates in low pH values, as standard methods of microfossil extraction employ acids for rock digestion. Consequently, the use of acids for clay-rich rocks is often inefficient and time-consuming. We propose a method of rock disintegration using the surfactant Rewoquat and compare it with two commonly applied approaches: digestion in buffered acetic acid and in HCl-HF. Using examples from the Mulde Brick-clay Member from the Silurian of Gotland and the Daleje Shale from the Devonian of the Prague Basin we observed that disintegration in Rewoquat was faster (days) than digestion in acid (months), and allowed to recover calcareous in addition to organic-walled fossils. The yield and preservation was comparably good, except for conodonts, which were strongly etched after using HCl-HF. Retiolitid graptolites recovered using Rewoquat were preserved in 3D and showed a lower degree of fragmentation. The fossil content of the residue obtained using Rewoquat was higher due to dispersion of clay aggregates. For observation of delicate fossils we recommend to coat the sample with the surfactant. Application of Rewoquat can reveal the most delicate forms and growth stages, and thus provide a better insight into the ontogeny, autecology, and body size distributions of a number of fossil groups.

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INTRODUCTION

Most techniques of microfossil extraction rely on acid digestion (e.g., Upshaw et al., 1957; Schopf, 1965; Miller, 1967; Chauff, 1978; Grahn and Afzelius, 1980; Jeppsson et al., 1985, 1999; Etherington and Austin, 1993; Jeppsson and Anehus, 1995; Ford and Lee, 1997) or rock expansion through freeze-thaw (e.g., Hanna and Church, 1928; Pojeta and Balanc, 1989; Remin et al., 2012). Highly argillaceous lithologies, such as claystones and marlstones, cause significant difficulties in the application of these methods due to their very low porosity and formation of clay coating on the sample surface. Yet, fine-grained rocks are an excellent source of well-preserved fossils, and are the most typical lithology of open shelf deposits, which are of great interest to biostratigraphers due to their stratigraphic completeness. In the present work, we propose a method of fossil extraction from clay-rich lithologies using the surfactant Rewoquat.

Rewoquat W 3690 PG is a trade name for a concentrate of 75% cationic surfactant 1-methyl-2-oleyl-3-oleyl-amidoethyl-imidazolium methosulfate with 24% propylenglycol. It is widely used for cleaning fossils (Lierl, 1992; Krüger, 1994; Riegraf and Niemeyer, 1996; Babinot and Colin, 2011). Less commonly, it is also used by foraminifer specialists for whole-rock disintegration (e.g., Holbourn and Kuhnt, 1998; Nagy, 2005; Heldt et al., 2008). However, we have not encountered any reports on the use of Rewoquat for extraction of phosphatic or organic-walled microfossils, to which belong the most important groups for Lower Palaeozoic biostratigraphy, i.e., conodonts and graptolites. In this study, we present the results of comparisons made between well-established methods of acid digestion and extraction using Rewoquat, applied to two Palaeozoic argillaceous samples: the Mulde Brick-clay Member from the middle Silurian of Gotland and the Daleje shale from the Lower Devonian of the Prague Basin. We demonstrate the advantage of surfactant-based rock disintegration with special focus being put on the recovery of Palaeozoic orthostratigraphic fossils, including graptolites, chitinozoans, and conodonts.

CASE STUDIES

Mulde Brick-clay Member, middle Silurian, Gotland

Sample Characteristics. We have used a sample from the middle Silurian Mulde Brick-clay Member (MBCM) of the Halla Formation. The uppermost part of MBCM is exposed in the Blåhäll 1 locality in western Gotland, Sweden (Hede, 1960; Laufeld, 1974a, 1974b; Calner et al., 2000, 2004a, 2004b).The MBCM represents an upper part of transgressive deposits of the intra- to peri-cratonic carbonate platform developed during the Early Palaeozoic on the East European Craton. They are characterized by a high content of argillaceous material and a very rich and diverse fauna (Calner et al., 2000, 2012), which includes calcareous fossils: brachiopods (e.g., Spjeldnaes, 1984; Wertel, 1995), ostracods (Martinsson, 1967), tentaculitids (Larsson, 1979), bryozoans, and a mass occurrence of trilobites (Calner et al., 2012); organic-walled fossils such as graptolites (Hede, 1942; Mierzejewski, 1988; Kozłowska-Dawidziuk, 1991; Bates et al., 2005), chitinozoans (Laufeld, 1974b), scolecodonts (Bergman, 1989); and (rare) conodonts (Jeppsson, 1983; Calner and Jeppsson, 2003).

Extraction

Two samples of 2 kg each, consisting of several boulder- and gravel-sized rock pieces, were used for comparison between acid dissolution and Rewoquat treatment.

TABLE 1. Number of chitinozoans and scolecodonts recovered from 10 g subsamples of the Mulde Brick-clay Member sample disintegrated using buffered acetic acid and Rewoquat (average of 3 replicates).

	Chitinozoans	Scolecodonts
Buffered acetic acid (7%)	319.4	97.7
Rewoquat	833.7	311

Disintegration in Rewoquat. For Rewoquat treatment, the sample was placed in a 20 L plastic bucket filled with 4 L of Rewoquat and tightly closed. The bucket was opened every other day to gently mix the content with a gloved hand. No hard objects were used to avoid crushing of fossils. After 10 days the bucket was filled with water and slowly poured in small portions over a stack of sieves with the following mesh sizes: 63 µm, 500 µm, and 1 mm. Sieving was performed with excess water, and the remainder of the extract in the bucket was diluted each time to reduce sieve clogging. Residues from each sieve were collected and, while wet, examined for floating fragments of retiolitid rhabdosomes. These have been collected with a pipette and stored in glycerin, then washed with water before transferring them onto SEM stubs. The residues were dried overnight at 30ºC, separated in sodium polytungstate to facilitate picking of phosphatic microfossils, and examined. Calcareous fossils were additionally cleaned in an ultrasonic bath for one hour. The extraction took 10 days of Rewoquat treatment and approximately 14 h of sieving.

Digestion in acetic acid. For acid dissolution, the sample was placed in extruded mesh (90–160 mm diameter) and hung in the upper part of a 30 L plastic bucket filled with 7% acetic acid buffered using the method of Jeppsson et al. (1999). The initial pH of the acid was 3.6. The bucket was tightly closed to prevent evaporation. Undissolved sample was rubbed gently every day to remove accumulated clay cover, which would prevent dissolution. Each time when the acid reached the pH of 4.6, it was exchanged, and the residue collected at the bottom was washed out, sieved over a stack of sieves (63 µm, 500 µm and 1 mm), and dried overnight in 30ºC. The bucket was re-filled with fresh buffered acid until the sample was completely dissolved. Only the 63-500 µm fraction was collected, but sieving was performed through a stack of sieves in order to disperse the water stream. The residues were dried overnight at 30ºC and separated in sodium polytungstate. The entire dissolution took 3.5 months.

In order to quantify the difference in microfossil recovery, 3 subsamples of 10 g each were retrieved from the acid- and Rewoquat-digested residues and the number of chitinozoans and scolecodonts (including fragmented specimens) was counted. The two groups were chosen as they were yielded in sufficient amounts by both methods and thus allowed a comparison. Average counts are given in Table 1.

Results

Recovered residues amounted to 202 g (10%) and 485 g (24%) for Rewoquat- and acid-extracted samples, respectively. The residue extracted with Rewoquat contained numerous rhabdosomes of the retiolitid graptolite *Gothograptus nassa* (Holm, 1890) preserved in three dimensions, exhibiting excellent preservation of cortical lists (Figure 1.5-8) and having partly preserved membranes (Figure 1.5-8). Recovered non-calcareous microfossils included chitinozoans (Figure 2.1-5), scolecodonts (Figure 2.6-10), two complete conodont elements (Figure 2.17-18), and one fragmented (not shown). The dominant proportion of the residue was formed by calcareous micro- and macrofossils, including tentaculites (Figure 3.1-3), tabulate corals (Figure 3.4-6), brachiopods (Figure 3.10-14), ostracods (Figure 2.12-14), echinoderms (Figure 2.11, 2.15-2.16), bryozoans (Figure 3.7), and trilobites (Figure 3.8-9), many of which bear encrustations from e.g., cornulitids (Figure 3.6-7), encrusting bryozoans, and other epibionts of unclear affinity, such as *Allonema* sp. (Figure 3.5, 3.8), *?Condranema parvula* (Condra and Elias, 1944) and *Ascodictyon venustum* Kiepura, 1965 (not shown).

The residue obtained with acid extraction consisted mainly of claystone fragments which hampered picking of fossils. Only chitinozoans and scolecodonts were obtained in large quantities (Figure 4). Calcareous fossils were absent, and retiolitid graptolites occurred only as small fragments. The average number of chitinozoans and scolecodonts in residue sample of the same weight was approximately one third of that obtained from the Rewoquat residue (38% and 31%, respectively; Table 1), mainly due to clay aggregates.

Transition layers between Zlíchov Limestone and Daleje Shale (Emsian, Lower Devonian, Barrandian)

Sample Characteristics. We have used samples from the Pekárek Mill section, located SW of Prague in the Švarcava Valley of the Prague Basin. The transition from carbonaceous sedimentation

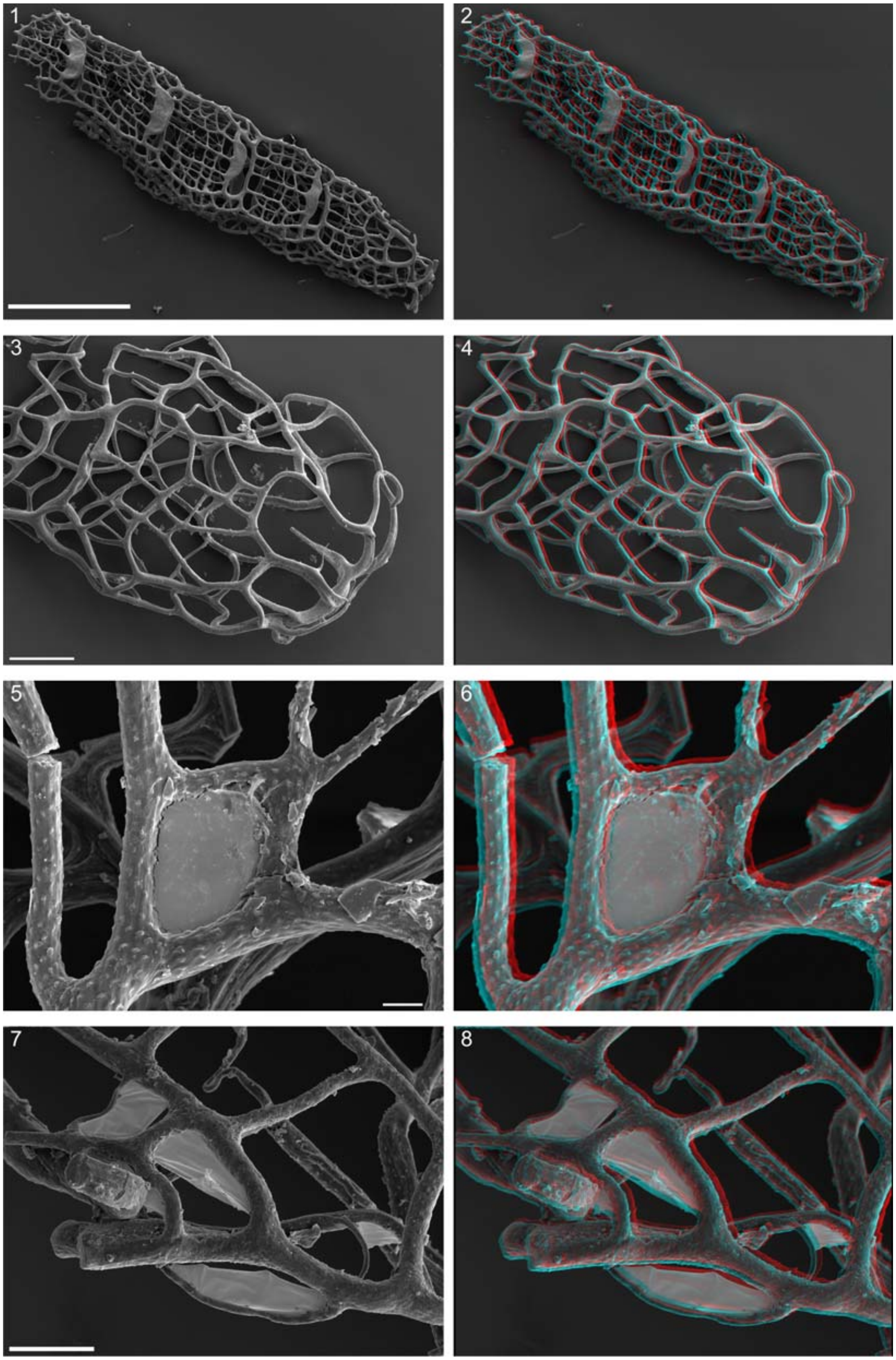

FIGURE 1. Retiolitid graptolite *Gothograptus nassa* (Holm, 1890) from the Mulde Brick-clay Member (Blåhäll 1, middle Silurian, Gotland), extracted using Rewoquat. 1-2 – ventral view of a rhabdosome with partly preserved proximal part, scale bar = 1 mm; 3-4 – ancora umbrella with preserved sicula, scale bar = 200 μm; 5-8 – membranes preserved between cortical lists, 5-6 scale bar = 15 μm, 7-8 – scale bar = 50 μm. 2, 4, 6, 8 – 3D anaglyphs.

FIGURE 2. Example fossils recovered with Rewoquat from the Mulde Brick-clay Member (Blåhäll 1, middle Silurian, Gotland): 1-5. Scale bar = 100 µm; 1. *Conochitina*? sp.; 2. *Conochitina tuba* Eisenack, 1932; 3. *Conochitina claviformis* Eisenack, 1931; 4. *Conochitina pachycephala*? Eisenack, 1964; 5. *C. pachycephala* Eisenack, 1964; 6. *Kettnerites martinssonii* Bergman, 1987, right first maxilla (MI); 7. paulinitid right MI; 8. deformed first paulinitid maxilla; 9. *Kettnerites* sp., left second maxilla (MII); 10. *Leptoprion*? sp., right MI; 11, 15-16. Echinoderm ossicles, 11 – scale bar = 200 µm; 16 – scale bar = 500 µm; 12 – *Aechmina* cf. *cuspidata* Jones and Holl, 1869; 13-15 – scale bar = 100 µm; 13 – *Hexophtalmoides* sp. Martinsson, 1963; 14. *Bollia* sp. Jones and Holl, 1886; 17-18 – scale bar = 200 µm; 17. *Panderodus unicostatus* (Branson and Mehl, 1933), simplexiform element, lateral view; 18. *P. serratus* Rexroad, 1967, costate element, lateral view.

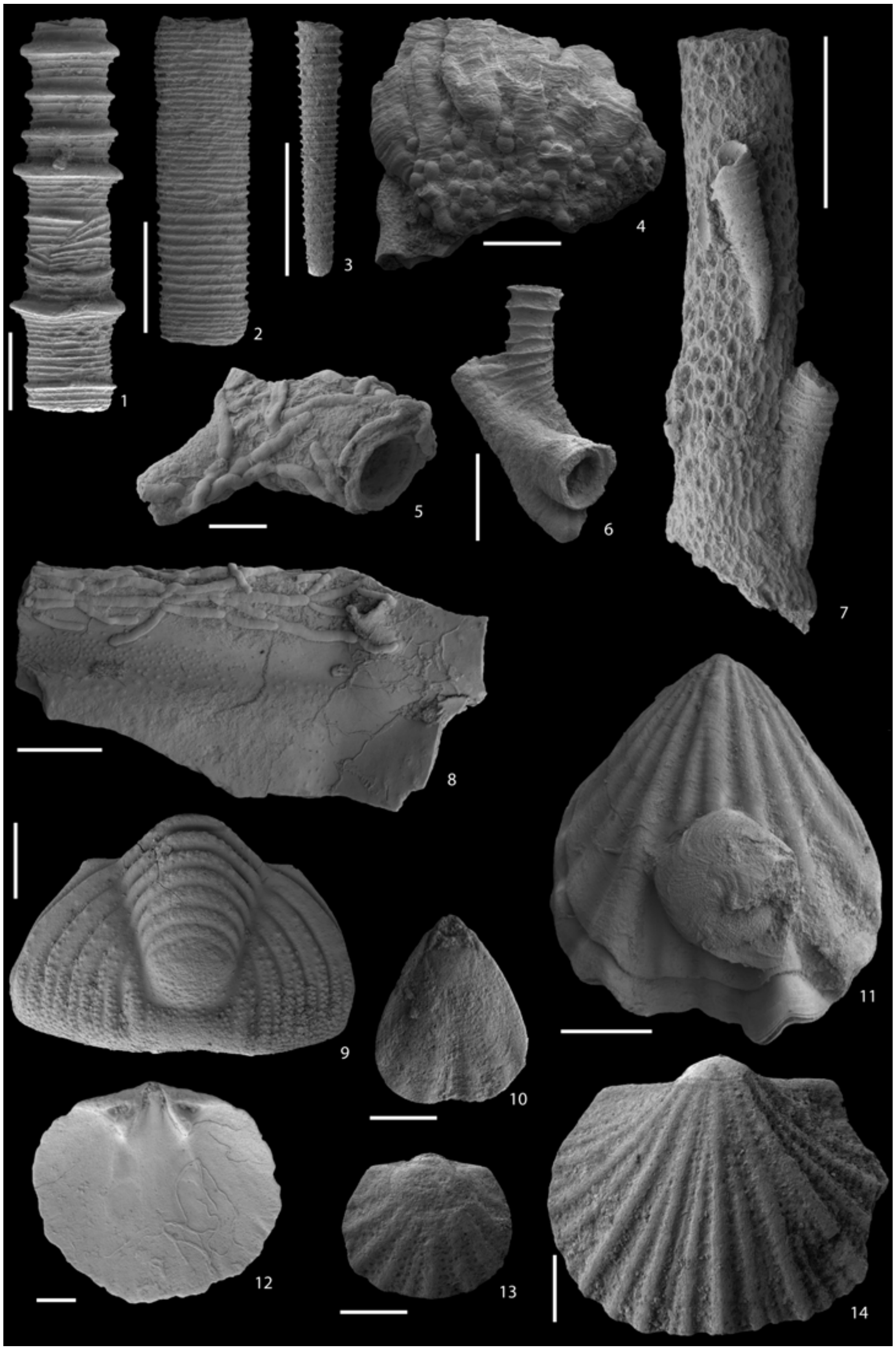

FIGURE 3. Example fossils recovered with Rewoquat from the Mulde Brick-clay Member (Blåhäll 1, middle Silurian, Gotland): 1. *Volynites muldiensis*? Larsson, 1979, scale bar = 500 µm; 2-3. juvenile parts of unidentified tentaculitoids, scale bar = 500 µm; 4. tabulate coral resembling *Planalveolites* sp.; 5. probable tabulate coral encrusted with *Allonema* sp., scale bar = 500 µm; 6. *Conchicolites* sp. encrusting a probable tabulate coral, scale bar = 1 mm; 7. fragment of a bryozoan colony encrusted by *Conchicolites* sp., scale bar = 2 mm; 8. trilobite genal? fragment encrusted with *Allonema* sp. and some unidentified encrusting organisms, scale bar = 1 mm; 9. trilobite pygidium, scale bar = 1 mm; 10, 13-14. unidentified juvenile brachiopods, scale bar = 200 µm; 11. *Stegerhynchus borealis*? encrusted with a microconchid or a juvenile *Anticalyptraea* sp., scale bar = 1 mm; 12. *Resserella* sp.? with encrustations, scale bar = 500 µm.

FIGURE 4. Example fossils recovered with buffered acetic acid from the Mulde Brick-clay Member (Blåhäll 1, middle Silurian, Gotland): 1-5. Scale bar = 50 μm for chitinozoans, 100 μm for scolecodonts; 1. *Conochitina claviformis*? Eisenack, 1931; 2. *Conochitina tuba*? Eisenack, 1932; 3. *Conochitina tuba*? Eisenack, 1932; 4. *Conochitina* sp.; 5. *Kettnerites* sp., right MII; 6. *Kettnerites martinssonii* Bergman, 1987, left MII; 7. *Conochitina pachycephala* Eisenack, 1964; 8. *K. martinssonii*, left MI; 9. *K. martinssonii*, right MI; 10. *K. martinssonii*, right MII.

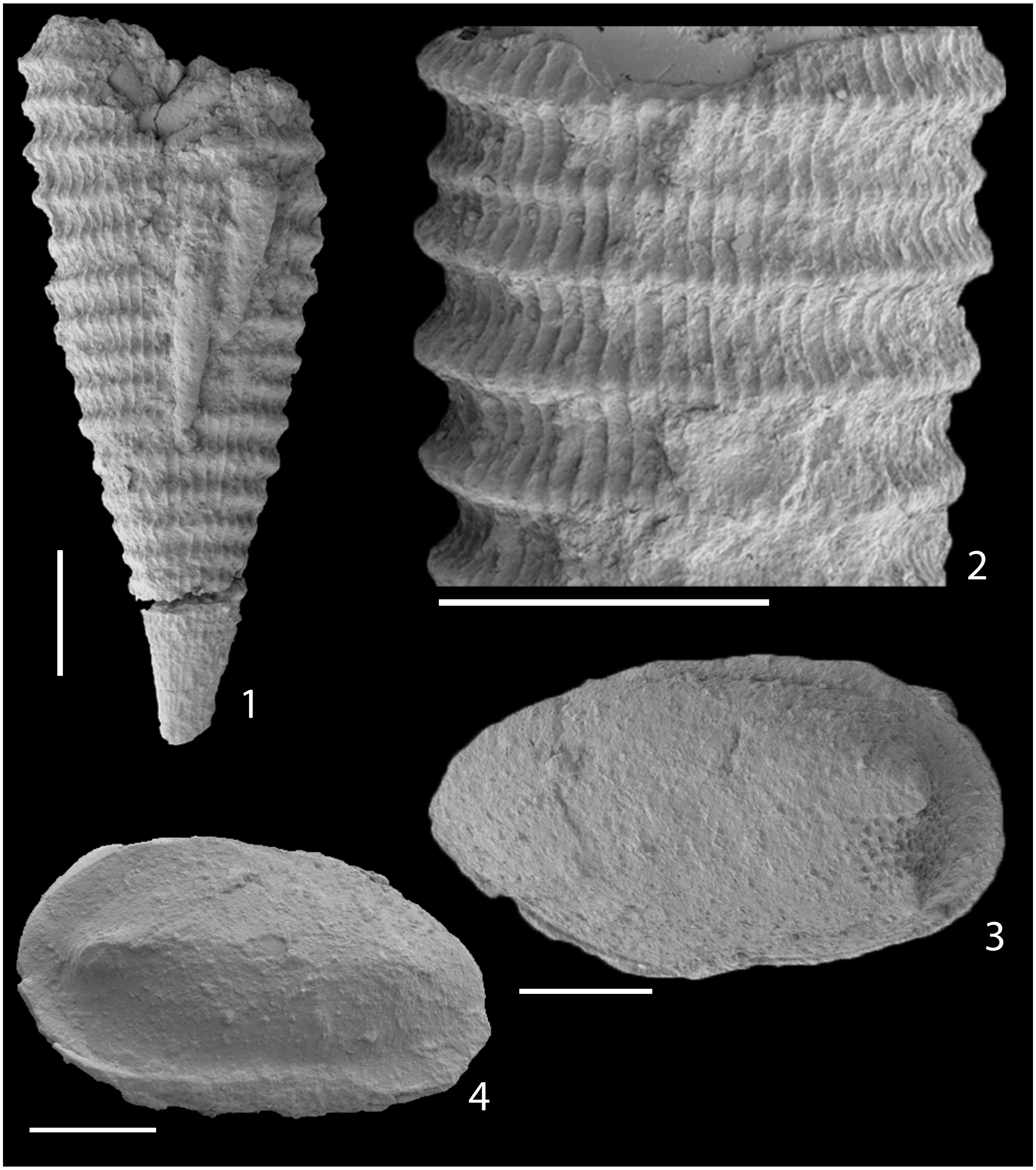

FIGURE 5. Example calcareous fossils recovered with Rewoquat from the Daleje Shale (layer CH15b, Pekárek Mill, Lower Devonian, Prague Basin): 1-2. *Nowakia barrandei* Bouček and Prantl, 1959, scale bar = 500 μm; 3. *Cribroconcha* sp. Cooper, 1941, scale bar = 200 μm; 4. healdiid ostracod, scale bar = 500 μm.

(Zlíchov Limestone) to calcareous shales (Daleje Shale of the Daleje-Třebotov Formation) reflects the onset of the Daleje Event (Chlupáč and Kukal, 1986; Ferrová et al., 2012). As the transition between the Zlíchov Limestone and the Daleje Shale is gradual, the predominance of argillaceous versus carbonate sedimentation is conventionally regarded as the lower boundary of the Daleje Shale. Pekárek Mill is well known due to common occurrence of dacryoconarid tentaculites (the Daleje Shale is referred to as “tentaculite shale”), trilobites, brachiopods, and, less frequent, but

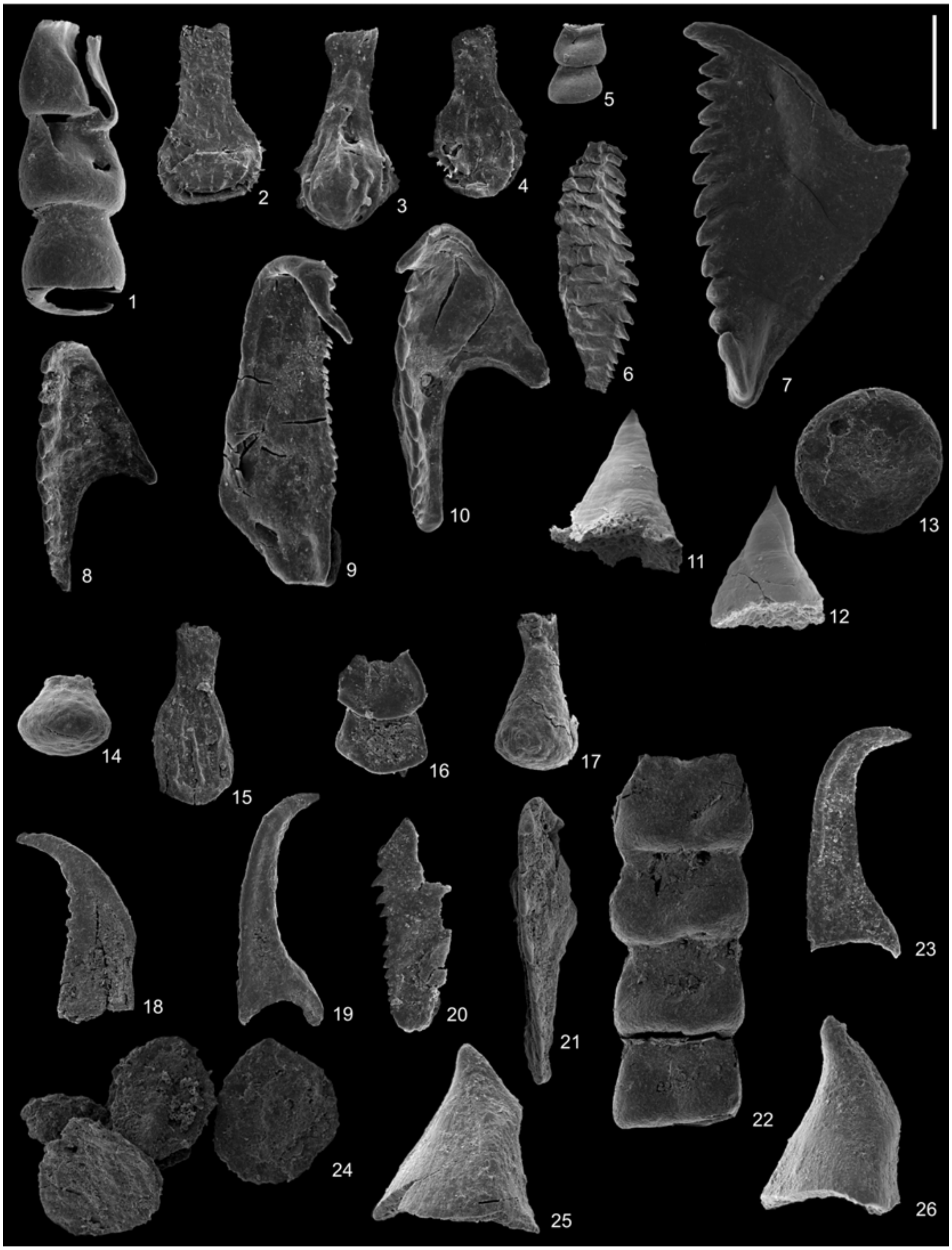

FIGURE 6. Example microfossils from the Daleje Shale (Pekárek Mill, Lower Devonian, Prague Basin). Scale bar = 100 µm, except for Figure 5.24, where the scale bar = 200 µm. Fossils in figures 1-13 were obtained using the HCl-HF-HCl technique and in figures 14-26 through disintegration with Rewoquat. 1. *Calpichitina*? sp., sample CH16b; 2-4. *Ramochitina* sp., 2. s. CH18b, 3. s. CH16b, 4. s. CH14b; 5. *Calpichitina* sp., s. CH16b; 6. *Lunoprionella* sp., s. CH18b; 7. polychaetaspid basal plate, s. CH16b; 8. *Kettnerites* sp., right second maxilla (MII), s. CH16b; 9. *Kettnerites* sp., left first maxilla (MI), s. CH16b; 10. *Kettnerites* sp., right MII, s. CH16b; 11. *Pseudooneotodus beckmanni* (Bischoff and Sannemann, 1958), s. CH15b; 12. *P. beckmanni*, s. CH14b; 13. unidentified prasinophyte, s. CH14b; 14. *Bulbochitina* sp., s. CH18b; 15. *Angochitina* sp., s. CH18b; 16. *Calpichitina* sp., s. CH16b; 17. *Angochitina*? sp., s. CH18b; 18. fragment of paulinitid MI, s. CH15b; 19. lateral tooth of Placognatha indet., s. CH15b; 20. Placognatha indet., s. CH17b; 21. *Oenonites* sp., s. CH17b; 22. *Calpichitina* sp., s. CH17b; 23. lateral tooth of Placognatha indet., s. CH16b; 24. prasinophytes, s. CH14b; 25. *P. beckmanni*, s. CH16b; 26. *P. beckmanni*, s. CH16b.

characteristic goniatites (Chlupáč, 1959; Chlupáč et al., 1979; Chlupáč and Lukeš, 1999). The petrography of the shales has been studied by Petránek (1950), who recorded higher $CaCO_3$ content linked especially to abundant faunal remains, particularly dacryoconarid tentaculites and calcified radiolarians. Detailed petrographic and chemical analyses and study of diagenetic pathways, however, have never been performed.

Extraction

For this study five shale samples were chosen from the interval CH14 to CH18 *sensu* Chlupáč and Lukeš (1999). Two samples of about 100 g have been used from each bed, one for the Rewoquat and the other one for acid treatment.

Disintegration in Rewoquat. Each sample was soaked in Rewoquat and left in a tightly closed preserving jar for 2—3 days, after that the remaining liquid was decanted, the jar was filled with water, and the sample was left to soak for at least one more day. Sieves with mesh sizes of 50 µm, 200 µm and 1 mm were used for all five samples. Ethanol was added during the sieving process to prevent foaming and to improve the cleaning of microfossils. The residues were dried at 60°C except for the smallest fraction (50—200 µm), which was partially picked in a wet state.

HCl-HF-HCl treatment. This technique represents a "classical" method for extracting organic microfossils (Schopf, 1965; Miller, 1967). The samples were treated with 35% HCl for three days. After dilution with water they were transferred into a plastic beaker and poured over with 40% HF (diluted to 30% by the remaining water) and left soaking until complete dissolution (for a week in this case), followed by the next dilution. In the last step, samples were boiled in concentrated HCl for a short (few minutes) time. Sieves of mesh sizes of 10 µm and 50 µm were used. The >50 µm fraction was hand-picked from a wet residue.

Results

Residues obtained by Rewoquat techniques consisted of abundant calcareous fossils such as dacryoconarid tentaculites (Figure 5.1-2), ostracodes (Figure 5.3-4), brachiopods, rugose corals, trilobites, palynomorphs including chitinozoans (Figure 6.14-17, 6.22), scolecodonts (Figure 6.18-21, 6.23), prasinophytes (Figure 6.24), and a small proportion of clay particles. Only one conodont species was recovered (Figure 6.25-26), all specimens displaying preservation of the outer hyaline tissue. Larger fossils (more than 5 mm) were often present as undeterminable scattered fragments. Some of the microfossils were covered with adherent particles (Figure 6.14-26), and therefore additional cleaning using hydrofluoric acid would be necessary.

The HCl-HF-HCl technique resulted in a residue composed almost solely of organic matter, consisting of acid-resistant microfossils: chitinozoans (Figure 6.1-5), scolecodonts (Figure 6.6-10), prasinophytes (Figure 6.13), and up to now undetermined palynomorphs of spore affinity). Conodonts were also recovered using this method, but the preservation was very poor, with the outer hyaline tissue entirely etched out (Figure 6.11-12). In processing of all five samples no indication of differences in mineralogical or physical properties was observed.

TABLE 2. Mineral composition of studied samples, determined using XRD analysis.

	Mulde Brick-clay Member	Daleje Shale
Illite	35.63%	24.76%
Clinochlore	4.47%	4.43%
Calcite	24.17%	41.82%
Dolomite	4.29%	-
Quartz	20.48%	22.25%
Albite	5.04%	5.91%
Illite/Smectite	1.29%	0.83%
Ankerite	4.09%	-
Pyrite	0.53%	-

MINERALOGICAL COMPOSITION

Sample preparation and measurement parameters

Small fragments (2 g) of matrix from each lithology, i.e., MBCM and the Daleje Shale, sample CH15b following the scheme of Chlupáč and Lukeš (1999), were ground in isopropanol using McCrone micronising mill with agate beads. Measurements of oriented samples mounted on flat plates were performed using Siemens D5000 theta-theta diffractometer with CuKα radiation, at 40 kV, 35 mA, graphite secondary monochromator, theta compensating slit, stepscan 0.02°, 3 sec counting time. Evaluation was performed using the Rietveld software "Topas".

XRD Results

The rock-forming clay mineral in the studied lithologies is illite, which consisted 35.63% of the MBCM sample and 24.76% of the Daleje Shale sample (Table 2). Both samples contained approximately 5% of clinochlore, also a phyllosilicate, and 20-23% of quartz. MBCM had a significantly smaller content of carbonate minerals (32.55% of calcite, dolomite and ankerite) than the sample representing the Daleje Shale (41.82% of calcite).

DISCUSSION

Comparison of Rewoquat- and Acid-based Extractions

The two described experiments were performed on samples representing highly clayey lithologies, from which the extraction of orthostratigraphic fossils poses a difficulty, both in terms of efficiency and time consumption. In both cases, extractions using Rewoquat yielded results after several (3-10) days, in contrast to up to 3.5 months of acid dissolution.

Organic-walled fossils (chitinozoans and scolecodonts) were obtained using both approaches, but, based on the MBCM sample, the amount of residue necessary to obtain a given number of specimens was approximately three times larger in the case of acid digestion, as the residue consisted of clay aggregates which likewise coated the fossils. The difference in recovery was pronounced with respect to retiolitid graptolites. We were able to extract long (up to 2 cm) fragments of rhabdosomes, including the proximal parts, which are most useful for taxonomic identification. On the contrary the buffered acid method yielded only small (<0.5 mm) rhabdosome fragments.

An obvious shortcoming of the acid treatment lies in the use of hazardous chemicals, especially hydrofluoric acid, whereas Rewoquat can irritate the skin and the eyes, but does not require a specialized laboratory to handle.

Extraction with Rewoquat allowed recovery of not only organic-walled, but also calcareous fossils. This is an advantage in situations where the available rock volume is limited, as it is in the case of drillcores, as well as when calcareous fossils, such as dacryoconarid tentaculites, can supplement biostratigraphic information obtained using non-calcareous groups. On the other hand, the method of whole-sample immersion with Rewoquat involves more intensive sieving in order to wash the chemical out of the residue, which incurs more human labour and a higher risk of breaking the most fragile fossils (e.g., trilobites). For such cases we recommend a cyclic procedure consisting of coating the sample surface by Rewoquat (see in the Recommendations). Argillaceous lithologies have been long recognized as the best sources of delicate fossils, such as graptolites (Barrande, 1850). Malformed and parasitized graptolite rhabdosomes, as well as their body parts which are normally not fossilized, are known virtually only from exceptionally preserved three-dimensional forms recovered from fine-grained sediments (Teller, 1998; Zalasiewicz et al., 2013). Extraction with Rewoquat from clay-rich rocks allowed us to observe a number of features important for the ontogeny and autecology, e.g., (1) the otherwise usually lost membranes between cortical lists of retiolitid graptolites, whose presence plays an important role in the reconstruction of retiolitid development (Kozłowska-Dawidziuk, 2004; Dobrowolska, 2012) (Figure 1.5-8); (2) well preserved protegula of juvenile brachiopods, the size and form of which is an indicator of the larval mode of life (planktotrophic vs. lecitotrophic, Freeman and Lundelius, 2007); (3) epibionts, which can be used as indicators of the growth habit of their hosts (Blind, 1970), and (4) shell repair marks in tentaculitoids, demonstrating the intensity of predation in these organisms with relatively poorly known ecology (Vinn, 2009, 2010).

Difficulties Arising due to a High Content of Phyllosilicates

In both discussed examples, the rock-forming clay mineral was illite, which is the dominant phyllosilicate in open shelf deposits. In acid solutions, illite displays a decrease in the apparent surface dissociation constant and forms aggregates (e.g., Jozefaciuk, 2002). Aggregation in low pH leads to coating the sample and clogging the pore space, which puts a rapid stop to further dissolution. This problem is well known to palaeontologists and archaeologists (e.g., Zhao and Pearsall, 1998; Lentfer and Boyd, 1999), and the only widespread approach is the use of dispersing agents whose action is based on replacement of cations in clay aggregates by hydrated sodium cations, which – due to their large hydrated radius – contribute to the dispersion of clay micelles (Zhao and Pearsall, 1998). These reagents include sodium hexametaphosphate (formerly referred to in the literature as Calgon, but note that the chemical sold under this trade name is not sodium hexametaphosphate anymore), sodium phosphate $[NaPO_3]_6/Na_2CO_3$, and Na_2H_2EDTA (Bates et al., 1978; Hodgkinson, 1991; Zhao and Pearsall, 1998). All these chemicals require numerous cycles of heating and sieving the released clay particles, which in marls need to be usually followed by another round of acid digestion. Grinding or shaking can improve deflocculation (Lentfer and Boyd, 1999; Coil et al., 2003), but is not applicable to brittle and delicate microfossils such as conodonts. In addition to significantly increasing the workload required for extraction, the use of sodium-based dispersing agents increases the mechanical stress applied to microfossils through multiple sieving and washing. Moreover, etching and formation of salt precipitates on the surface of microfossils have been reported in the literature for all these chemicals (Hodgkinson, 1991) and confirmed by our own (EJ's) observations. The time required for acid digestion of marlstones, even without the subsequent use of deflocculants, is measured in weeks to months, whereas in Rewoquat disintegration time is of the order of several (1-14) days depending on the degree of cementation.

Methodological Biases Arising from the Differences between Extraction Techniques

In all approaches to microfossil extraction the effects of chemical or mechanical treatment do not have the same effect on all fossils, e.g., in acid digestion smaller conodonts are selectively more vulnerable to dissolution (Jeppsson et al., 1985, 1999), and in the Glauber's salt method the plaktonic foraminifers are destroyed in a higher proportion than benthic forms (Remin et al., 2012). Therefore an accurate account of the extraction protocol and reduction in the number of individuals lost through crushing, dissolving, and etching is crucial in collecting biostratigraphic data and for their statistical handling (Jeppsson, 2005). The bias introduced through selective destruction or inefficient extraction of the smallest and most fragile forms has a number of paleoecological and biostratigraphical implications (e.g., Jeppsson, 1997, 2005; McGowan et al., 2009). For instance, different conodont elements belonging to a single species are usually not recovered with expected frequencies. This is usually attributed to taphonomic processes (Bitter and Purnell, 2005; Purnell and Donoghue, 2005), but laboratory processing leading to the loss of smaller elements can enhance this disparity, and even completely obliterate the presence of the most fragile taxa (Jeppsson, 1997).

In addition to the importance for biostratigraphic and ontogenetic studies, detection and extraction of smallest individuals is essential in reconstructing the development of body size distributions. Intermittent shifting of body size distributions towards dwarf forms (the 'Lilliput effect', Urbanek, 1993) has been discussed for a number of taxonomic groups, e.g., during major paleoecological crises (Payne, 2005; He et al., 2007; McGowan et al., 2009; Posenato, 2009; Brayard et al., 2010; Payne et al., 2011; Sigurdsen, 2012). In the Silurian Period, the tendency to micromorphism also has been observed with respect to conodonts (Slavík et al., 2010), graptolites (Urbanek, 1993), and in brachiopods (Erlfeldt, 2006; Pakhnevich, 2009), and these trends have commonly been linked to unfavourable environmental conditions. Brachiopod populations inhabiting soft-bottom, marly habitats, and, in general, deeper waters, are considered to develop smaller body sizes (Bitner, 2002; Peck and Harper, 2010). As many studies are based on the collection of brachiopod specimens from bedding planes (e.g., Wertel, 1995; Pakhnevich, 2009), the proportion of micromorphic brachiopods may be easily underestimated. In the present study, from the MBCM sample we were able to recover brachiopod shells as small as 450 µm in length, and we suggest that the employment of Rewoquat may help in improving the representation of body-size trends of brachiopods and other groups of organisms with calcareous skeletons, e.g., trilobites and echinoderms, in which micromorphism has been less recognized. Rewoquat is efficient with most argillaceous, non-cemented lithologies, which have been shown to generally preserve higher biodiversity than cemented rocks, both due to taphonomic processes in the sediment, and to methodological problems in extracting fossils from indurated rocks (Hendy, 2009; Sessa et al., 2009; Hendy, 2011; Nawrot, 2012).

RECOMMENDATIONS FOR EXTRACTIONS WITH REWOQUAT

Disintegration should be performed on a dry sample and take place in a closed container to prevent evaporation. Broad, flat containers and gentle crushing of the sample with bare hands will increase the surface contacting with the reagent. Stirring with a soft object (e.g., a gloved hand or wooden stick) can speed up disintegration. Once disintegration comes to an end, Rewoquat should be decanted, the residue diluted with water and left to soak for at least one more day in a closed container. The decanted reagent can be recovered through decantation and sieving over a 63 µm mesh; the resulting reagent will contain clay particles, but it still can be used several times to recover larger fractions. The residue can be sieved in small portions and washed with a water brush. Ethanol can be added to reduce foaming. The procedure can be adapted for extraction of a particular group of fossils, e.g., for retiolitid graptolites the sieving and water brushing steps should be reduced to avoid fragmentation of rhabdosomes, and for delicate fossils supported by matrix, such as trilobites or articulated echinoderms, we recommend to coat the sample using a brush and hermetically close it in a plastic container. Following disintegration it can be handled in the same way as described above, with the remaining large pieces washed separately. They can be studied under the microscope for fragile fossils, which remain supported by the matrix. Rock pieces larger than 200 µm should be coated with Rewoquat again and the procedure repeated. This method allows release of the rock matrix with microfossils and examine delicate calcareous structures in the same time.

Disintegration in Rewoquat can be included in a multi-step procedure, e.g., to remove the clay and increase the effective porosity of a carbonate- or silica-cemented rock sample, which can be further subjected to acid dissolution or etching.

Rewoquat is inflammable and may irritate the skin (Xi R36/38). It should be stored in air-tight containers. Gloves and goggles are necessary (also during sieving and washing), and handling of samples in Rewoquat should, if possible, take place under a fume-cupboard.

CONCLUSIONS

1. Microfossil extraction in the organic surfactant Rewoquat W 3690 PG was up to 10 times faster than acid dissolution when applied to two highly clayey rock types, thanks to preventing formation of clay aggregates. In both cases, Rewoquat allowed to recover calcareous fossils which were lost in acid extraction.
2. We recommend the use of Rewoquat in microfossil extraction from marls and claystones. The method does not work for firmly cemented rocks such as silicaceous shales or dolostones.
3. Extraction with Rewoquat is performed by soaking the sample in the surfactant in a sealed container. Following complete disintegration the remaining Rewoquat can be recovered by decantation, the residue should be then soaked with water for one more day and then sieved with excess water.
4. Rewoquat does not dissolve or etch fossils. It allows to recover all types of microfossils, e.g., conodonts, retiolitid graptolites, chitinozoans, foraminifers, ostracodes, scolecodonts, etc. However, matrix-supported fossils such as trilobites may fall apart upon rock disintegration in Rewoquat; we recommend that in such cases Rewoquat should be used to clean them on a rock bedding plane.

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