

# An experimental evaluation of cleaning methods for fossil ostracod Mg/Ca and Sr/Ca determination

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**Abstract** To evaluate the effect of sample preparation on the trace element composition of ostracod shells, fossil shells subjected to various cleaning steps were analyzed by ICP-AES. The variations in ostracod Mg/Ca ratios reveal that fossil ostracod samples cleaned by ultrasonic methanol yield lower values than those by other methods, including increasingly rigorous reductive cleaning. In our sample sets, shell Mg/Ca decreases ~1.5 mmol/mol during clay removal. Sample Sr/Ca results do not seem to vary significantly under the equivalent cleaning steps. For ostracod analysis, pre-treatment using multiple methanol ultrasonic cleaning is suggested to be used for gaining an appropriate result.

**Keywords** Ostracod · Cleaning experiment · Trace element · ICP-AES

## Introduction

Trace element ratios (Sr/Ca and Mg/Ca) and stable isotope measurements ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) on ostracod shell calcite provide a useful tool in the reconstruction of Quaternary paleoclimate change giving paleosalinity and paleotemperature in terrestrial aqueous environments (e.g., Lister et al. 1991; Wells et al. 1999; Schwalb 2003; Dettman et al. 2005; Palacios-Fest et al. 2005; Wetterich et al. 2005). Sample preparation of ostracod tests is a vital stage of shell chemical analysis because different methods will drastically affect the resolution and reproducibility, and inter-laboratory comparison.

The first challenge of ostracod sample preparation for providing quantitative information on shell chemistry is to remove any adherent surface-bound contamination. Such contamination may include clays, organic matter, Fe–Mn oxides and other particles. Baker et al. (2003) evaluated the effects of a series of cleaning steps to trace element measurements of oceanic foraminiferal samples, however, little detailed investigation into these relative effects on ostracod samples has been carried out (Holmes 1992), although both are calcifying organisms. This study evaluates the most

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popular and frequently performed techniques in physical/chemical cleaning of shells prior to geochemical analysis.

A fine-hair brush wetted with deionised water was used to manually remove particles under a binocular microscope (Holmes 1992). Additional chemical procedures used to remove organic matter and/or clay particles include boiling in dilute H<sub>2</sub>O<sub>2</sub> (e.g., von Grafenstein et al. 1994; Schwab et al. 1994; Hu et al. 1998), treating with ethanol (e.g., McCulloch and De Deckker 1989) and heating either in an oxygen plasma (e.g., Boomer 1993) or in a vacuum (e.g., Lister et al. 1991). Until now, different groups employ a diverse range of sample preparation and pre-treatment techniques, leading to difficulties in comparing results between laboratories.

Obviously, there is an urgent need to develop a standard cleaning or pre-treatment procedure for ostracod analysis, similar to that now commonly used in foraminiferal studies. In this paper, we present the results of a series of cleaning experiments and evaluate their effect on Mg/Ca and Sr/Ca analyses.

### Sample preparation and cleaning procedure

The ostracod species we used for the cleaning tests were *Qinghaicypris* Huang 1979 and *Psychrodromus* Danielopol & McKenzie 1977 from a 197 m long lake sediment core at Co Ngoin (CE-1, 31°31'18"N, 91°32'55"E), central Tibetan Plateau. The *Psychrodromus* Danielopol & McKenzie specimens were from the 141.16–141.06 m interval and three *Qinghaicypris* Huang samples were from the 142.00–141.90 m, 81.20–81.10 m and 71.05–70.95 m intervals of core CE-1, respectively. We chose these specimens because they (i) exhibit good preservation, with no visible dissolution after burial and no calcite overgrowth, (ii) are abundant and (iii) have thick valves. In our tests, adult and A-1 instar valves of the specimens were used for obtaining consistent trace element compositions (Mg, Sr) for each group sample and for evaluating the effectiveness of cleaning, because juvenile ostracods can incorporate an anomalously high Mg contents and anomalously low proportions of Sr in their shells (Griffiths and

Holmes 2000). Some thin shells are easy to crack during brush cleaning, thus we used the relatively large with thickly calcified shells to avoid excessive loss of samples during cleaning. The weight of each single valve is 85–100 µg for A-1 and 105–115 µg for adult of *Psychrodromus* Danielopol & McKenzie, and 138–145 µg for A-1 and 155–166 µg for adult of *Qinghaicypris* Huang. Two valves were used for each treatment and analyses in our tests.

All hand-picked ostracod shells from pre-sieved lake sediments were initially cleaned thoroughly using a UHQ (ultra high quality) deionised water moistened brush to remove visible particles. In contrast to foraminiferal tests, it is not necessary to crush the valves in order to open the carapace. However, a fine paint brush was used to remove particles within lips, canals and small pores. Particulate removal at this stage was time-consuming. After manual brush cleaning and weighing, two shells for one test were transferred to an acid cleaned 500 µl micro-centrifuge Eppendorf tube using a moistened brush.

Group samples were then subjected to each of the following additional cleaning methods (Table 1), including ultrasonic UHQ water cleaning (Method A), hydrogen peroxide treatment (Method B), hydroxylamine hydrochloride treatment (Method C), sodium dithionite treatment (Method D) and ultrasonic methanol cleaning (Method E). Some samples were cleaned thoroughly using a methanol-moistened brush for comparison. Repeated treatment was used and batch treatment for ultrasonication was appropriate.

Due to the open chamber, some ostracod shells may float on the water surface while adding and removing reagents, especially during methanol treatment. To avoid loss of shells from each tube by pipette at each stage, flicking the tube with a fingernail will help all test shells settle to the tube bottom. After the overlying solution was removed individually, multiple UHQ H<sub>2</sub>O washes were then employed to remove suspended impurities and any remaining reagent after each treatment. After each any treatment, samples were dried in a drying cabinet and reweighed. The cleaned samples can be stored in a fridge until dissolved and analyzed.

**Table 1** Cleaning methods and procedures for the preparation of ostracod shells in our experiments

Method	Aim	Procedure description	Reagents	Source
Brush cleaning	Removal of visible particles	Initially all shells were cleaned thoroughly under a binocular microscope using a fine-hair brush		
Method A	Clay removal	Squirt 500 µl of UHQ H <sub>2</sub> O onto the test sample Flick the tube with a fingernail help shells settle to the tube bottom Place the sample rack in an ultrasonic bath for 1–2 min Remove the overlying solution with a separate pipette	UHQ H <sub>2</sub> O UHQ H <sub>2</sub> O	Holmes 1992 Baker et al. 2003
Method B	Removal of organic matter	Add 250 µl of alkali buffered 1% H <sub>2</sub> O <sub>2</sub> solution to each tube and secure the rack with a lid to prevent tubes popping open while under pressure Place the sample rack in a boiling water bath for 10 min Remove the rack and rap on the bench top to release any gaseous build-up after 2.5 and 7.5 min, after 5 min place the rack in an ultrasonic bath for a few seconds and returned to the water bath after rapping on the bench. The aim of these interim steps is to maintain contact between reagent and sample	30% H <sub>2</sub> O <sub>2</sub> 0.1 M NaOH	von Grafenstein et al. 1994; Baker et al. 2003
Method C	Removal of Fe–Mn oxides	Remove the oxidizing reagent using a pipette Treat the samples with a standard hydroxylamine hydrochloride solution (25 g NH <sub>2</sub> OH·HCl + 300 ml UHQ H <sub>2</sub> O + 200 ml conc. NH <sub>3</sub> ) at 80°C for 10, 20, and 30 min Treat with a basic sodium dithionite (25 g NH <sub>2</sub> OH·HCl + 300 ml UHQ H <sub>2</sub> O + 200 ml conc. NH <sub>3</sub> + 25 g citric acid solution with 0.5 g Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> add to 10 ml of this mixed reagent just before use) at 80°C for 10, 20, and 30 min Draw 250 µl methanol off the sample with a pipette	NH <sub>2</sub> OH·HCl NH <sub>3</sub> UHQ H <sub>2</sub> O NH <sub>2</sub> OH·HCl NH <sub>3</sub> Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> UHQ H <sub>2</sub> O	Boyle 1981 Boyle 1981
Method D	Removal of Fe–Mn oxides			
Method E	Clay removal	Place the sample rack in an ultrasonic bath for 1–2 min Inject straight back in to bring clays into suspension and remove the reagent using a pipette	Aristar® methanol	Baker et al. 2003; Our test

## Dissolution and ICP-AES analysis

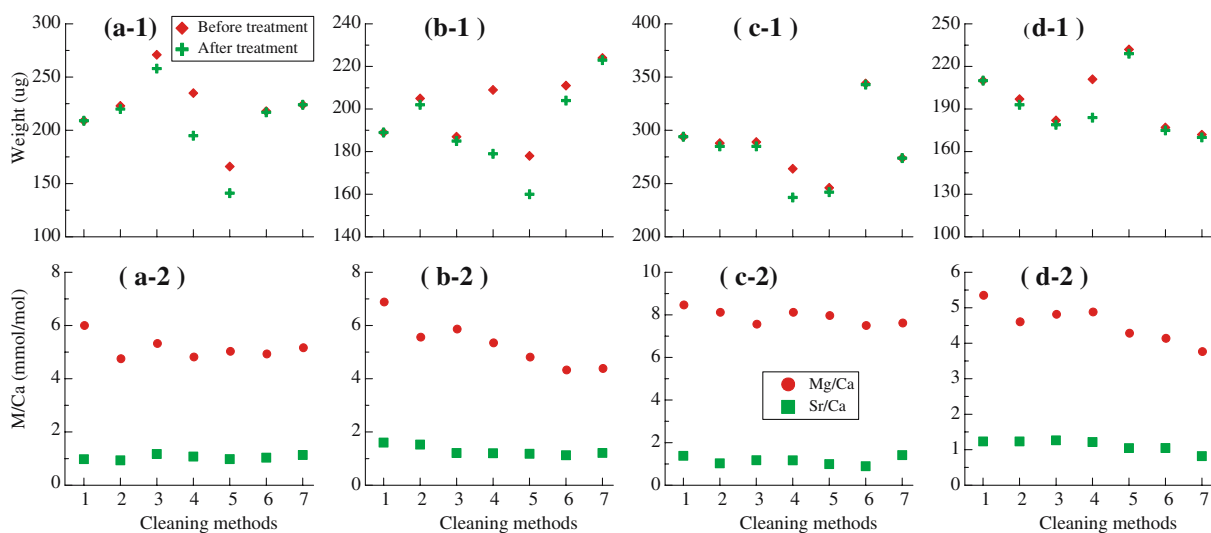
After adding 350  $\mu\text{l}$  (500  $\mu\text{l}$  for large samples) of 0.075 M  $\text{HNO}_3$  to each sample, the sample rack was placed in an ultrasonic bath to promote reaction. Every few seconds, each tube was tapped with a fingernail to allow any build-up of  $\text{CO}_2$  to escape and the reaction to continue. As soon as production of  $\text{CO}_2$  ceased in any sample tube, the tubes were removed from the ultrasonic bath and left to settle. Then 300  $\mu\text{l}$  of solution was immediately transferred to a clean sample tube, leaving any particles to be discarded in the residual 50  $\mu\text{l}$ .

To gain the best precision and accuracy of Mg/Ca and Sr/Ca determinations using an ICP-AES, solution containing 60 ppm Ca concentration was used (de Villiers et al. 2002). An initial concentration determination from a 5-fold dilution was followed by a second run at optimum Ca concentration for Mg/Ca and Sr/Ca ratios. Trace element analyses was carried out on a Varian Vista axially viewed plasma (AX) charge coupled device (CCD) simultaneous ICP-AES using an intensity ratio calibration outlined by de Villiers et al. (2002) in the Department of Earth Sciences, University of Cambridge, with an average precision of 0.2%.

## Results and discussion

To test and verify of replicability of measured results, duplicate measurements at each level were used to calculate a statistically valid estimate of geochemical variability within ostracod samples by many researchers. However, similar values were obtained when the same cleaning method was used, but this does not always represent appropriate values of carbonate samples. On the other hand, insufficient shells mean that it is not possible to carry out enough duplicate measurements for the comparisons to be statistically significant, especially when multiple-proxy analysis is undertaken on each sample, reinforcing the need for an appropriate standard pre-treatment of ostracods.

Variations in shell weight, Mg/Ca and Sr/Ca values for fossil ostracods treated by each single cleaning method were shown in Fig. 1. The shell weight varied most in the treatment by hydroxylamine hydrochloride solution (Method C) where shell weight was 10–14% lower than that of those subjected to manual brush cleaning alone. Mg/Ca ratios of all sample sets subjected to different cleaning methods were also lowered compared to manual brushing. The most significant reduction



**Fig. 1** Comparison of single cleaning method on shell weight, Mg/Ca and Sr/Ca ratios. (a)–(c) *Qinghaicypris* Huang, (d) *Psychrodromus* Danielopol & McKenzie.

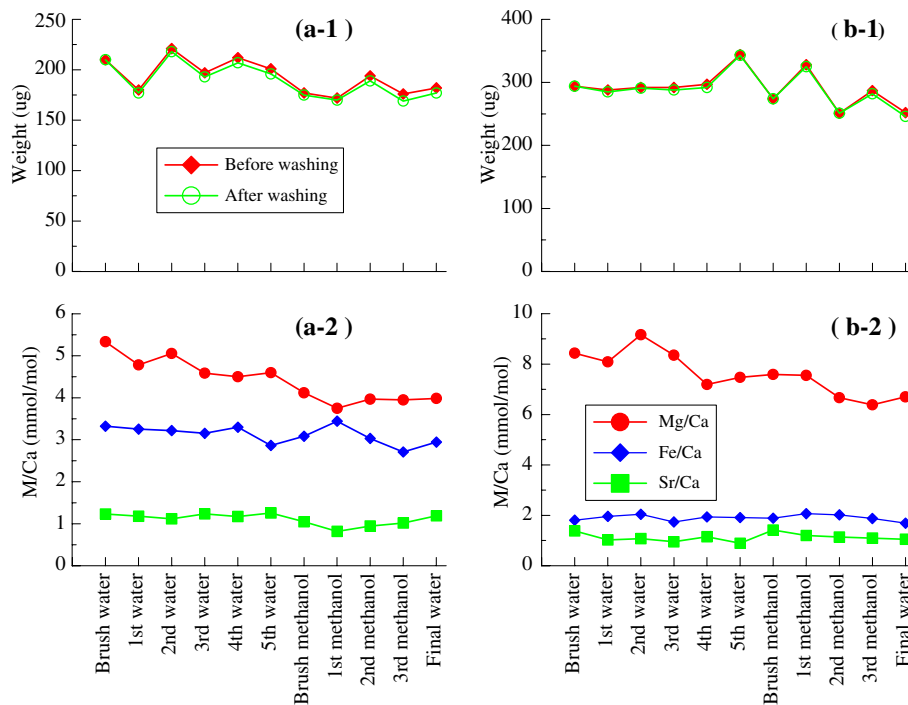
Cleaning methods: 1. Brush water, 2. Method A twice, 3. Method B 20 min, 4. Method C 20 min, 5. Method D 20 min, 6. brush methanol, and 7. Method E 20 min

in Mg/Ca values was in the shells subjected to the ultrasonic methanol bath (Method E), while hot 1% H<sub>2</sub>O<sub>2</sub> (Method B) and sodium dithionite (Method D) reduced the values as well (Fig. 1). The various cleaning methods have lesser effect on Sr/Ca ratios in most tested samples than on Mg/Ca ratios (Fig.1). In the samples we tested, though there should be systematic offsets within samples and species, co-variations between four sample groups should be attributed to removal of Mg contaminants associated with fine clay particles and/or organic matter. It indicates ~30% (1.6 mmol/mol for *Psychrodromus* Danielopol & McKenzie) and 10–36% (0.8–1.5 mmol/mol for *Qinghaicypris* Huang) lower Mg/Ca values for the samples cleaned by the ultrasonic methanol (Method E) than those by manual brush.

To test the effect of contaminant removal on trace element composition, fossil samples were analyzed after step-by-step UHQ water and methanol cleaning. Fig. 2 shows the considerable cumulative effect of contaminant removal on sample weight and Mg/Ca ratios for both species. The Mg/Ca values are not lowered any further by

multiple-step cleaning, especially after Method E. Both ultrasonic baths of UHQ water and methanol are effective methods for removing clay particles, thus we suggest that clay particles have the most important effect on Mg/Ca values. The effect of clay contamination on ostracod Mg/Ca values may differ significantly from various species (Griffiths and Holmes 2000) and locations. Ostracod shells with lips and small pores and those with low Mg/Ca ratios, for example, may be more prone to significant contamination than those with higher Mg concentrations.

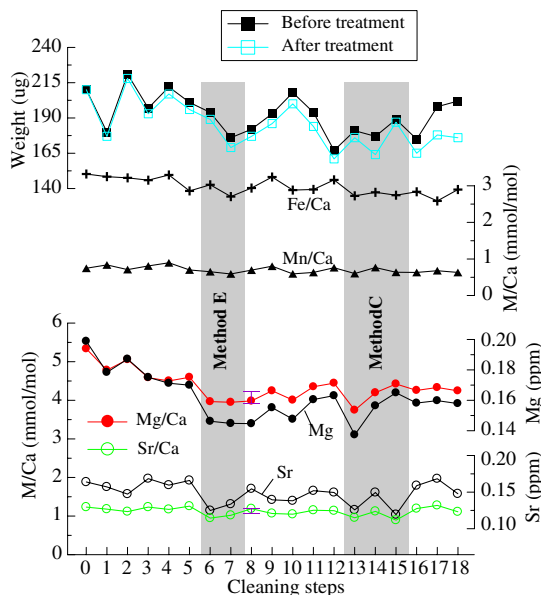
Further cleaning steps were taken for treating eighteen *Psychrodromus* Danielopol & McKenzie samples to refine clay removal, as listed in Table 2. Results show that there is no obvious offset in the measured shell Mg/Ca ratios after UHQ water and methanol cleaning, even when subjected to increasingly rigorous reductive cleaning stages (Methods C and D) (Fig. 3). Assuming Mg comes from Fe–Mn oxide coating present as in foraminiferal samples (Barker et al. 2003), the samples treated by reductive cleaning would be expected to show a decrease in Mn/Ca and Fe/Ca as well as



**Fig. 2** The effect of clay removal on ostracod sample weight, Mg/Ca, Sr/Ca and Fe/Ca ratios. (a-1 and a-2) *Psychrodromus* Danielopol & McKenzie, (b-1 and b-2) *Qinghaicypris* Huang

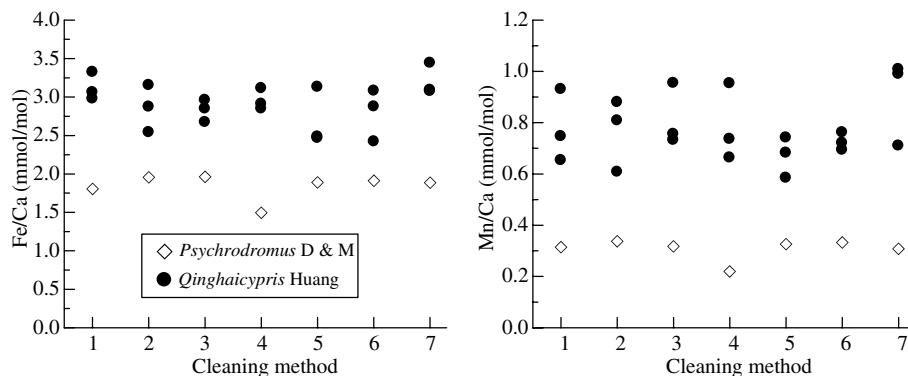
**Table 2** The complete cleaning steps for *Psychrodromus* Danielopol & McKenzie shells

Steps (series number in Fig. 3)	Cleaning method
0	Brush UHQ H <sub>2</sub> O cleaning
1	1 time UHQ H <sub>2</sub> O ultrasonic bath (Method A)
2	2 times UHQ H <sub>2</sub> O ultrasonic bath (Method A)
3	3 times UHQ H <sub>2</sub> O ultrasonic bath (Method A)
4	4 times UHQ H <sub>2</sub> O ultrasonic bath (Method A)
5	5 times UHQ H <sub>2</sub> O ultrasonic bath (Method A)
6	Plus 1 time methanol ultrasonic bath (Method E)
7	Plus 2 times methanol ultrasonic bath (Method E)
8	Plus final UHQ H <sub>2</sub> O ultrasonic bath (Method A)
9	Plus 10 min H <sub>2</sub> O <sub>2</sub> oxidative treatment (Method B)
10	Plus 20 min H <sub>2</sub> O <sub>2</sub> oxidative treatment (Method B)
11	Plus 30 min H <sub>2</sub> O <sub>2</sub> oxidative treatment (Method B)
12	Plus 40 min H <sub>2</sub> O <sub>2</sub> oxidative treatment (Method B)
13	Plus 10 min reductive treatment (Method C)
14	Plus 20 min reductive treatment (Method C)
15	Plus 30 min reductive treatment (Method C)
16	Plus 10 min reductive treatment (Method D)
17	Plus 20 min reductive treatment (Method D)
18	Plus 30 mins reductive treatment (Method D)



**Fig. 3** The effect of cleaning methods (Table 2) on weight ( $\mu\text{g}$ ), and Mg, Sr concentrations and their ratios with Ca of *Psychrodromus* Danielopol & McKenzie samples. The error bars of Mg/Ca and Sr/Ca ratio show the measured value range by using our recommended cleaning method

Mg/Ca, even if not all coating was removed by reductive cleaning. However, no significant decrease was seen in the Fe/Ca and Mn/Ca ratios for the treated ostracod samples, even treated by Methods C and D (Figs. 3 and 4). Similarly, if the observed step decrease in Mg/Ca seen for ultrasonic UHQ water and methanol washes was associated with removal of Fe–Mn oxide coating, we may expect a stepwise decrease in Fe/Ca. This is further supported by observation that partial



**Fig. 4** Comparison of single cleaning method on Fe/Ca and Mn/Ca ratios of both *Qinghaicypris* Huang and *Psychrodromus* Danielopol & McKenzie. Cleaning methods are in the same order as Fig. 1

dissolution of shell calcite caused by rigorous reductive cleaning (Method D) did not show a decrease in both Mg/Ca and Fe/Ca (Fig. 3), and that Fe/Ca ratios in the tested samples have good correlation with Mn/Ca values, but not with Mg/Ca ratios (Fig. 5). The findings show that Mn–Fe oxide coating has less relation with contaminant Mg concentrations. This would suggest that both Fe and Mn were incorporated proportionally into the lattice as the ostracod shell formed. Thus, the best way to decrease the contribution of Mg from

surface-bound contaminants to the corresponding Mg/Ca ratio is clay removal. Sample preparation for Sr/Ca analysis does not require as many cleaning steps as that for Mg/Ca.

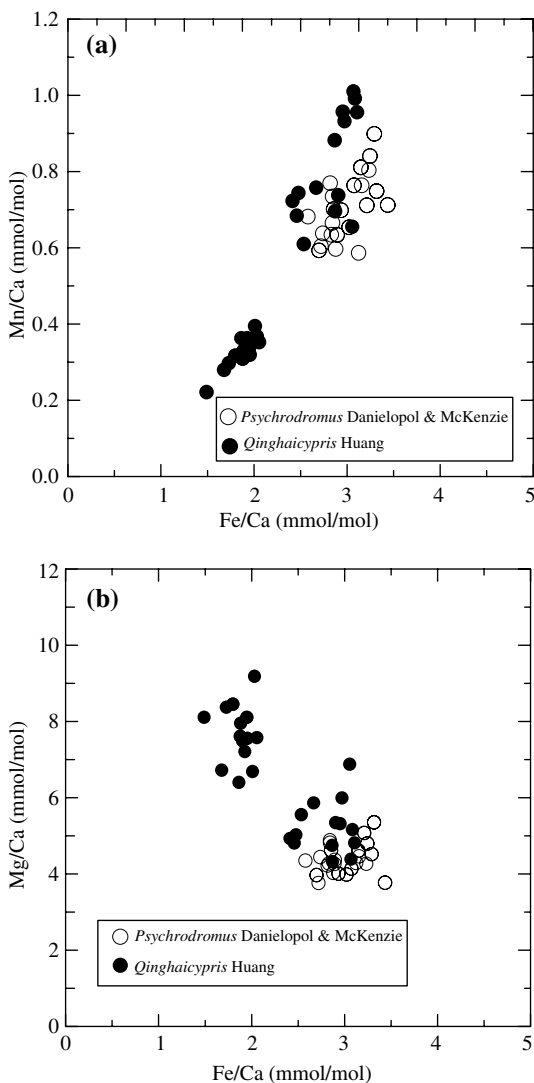
### Suggested cleaning method

We suggest that manual cleaning with a fine-hair brush, commonly used before ostracod analyses, might yield significantly higher measured Mg/Ca ratio than the original value of the pure shell carbonate lattice. After manual brush cleaning, use of ultrasonic methanol and deionized water cleaning steps is necessary. It is apparent that clay removal has, by far, the most significant influence on Mg/Ca value of ostracod sample. In clay removal for ostracod analyses, multiple-step ultrasonic methanol cleaning provides the most effective method. After any methanol wash, further UHQ water should be used in order to remove any remaining methanol. To reduce the risk of contamination from any non-carbonate particles that may still be present in the samples, centrifuging (500 rpm for 5 min) is necessary to settle any remaining small silicate particles to be removed after dissolution. Cleaning steps can be varied according to ostracod species and sediment nature. To develop a standard cleaning protocol, similar to that now used in foraminiferal preparation, more cleaning experiments would be required to include differing location, species, time periods and types of sediment matrix, as suggested by Holmes (1992).

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**Fig. 5** Comparison of Fe/Ca versus (a) Mn/Ca and (b) Mg/Ca ratios obtained by use of different cleaning methods of both *Qinghaicypris* Huang and *Psychrodromus* Danielopol & McKenzie samples

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