ACID PREPARATION OF LARGE VERTEBRATE SPECIMENS

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Acid preparation of large vertebrate fossils poses special problems for the preparator. The Fundación Colombiana de Geobiología has prepared a number of large vertebrates (marine reptiles from the Cretaceous of Colombia, South America) using acid to remove calcareous matrix. A combination of factors, including: specimen size: choice of acid; number and length of acid baths; ventilation needs; area of matrix and fossil exposed; matrix homogeneity; number of acid resistant protective coats applied; management of voids; and acid consumption are shown to be important. By varying these parameters, exceptional preparation of specimens ready for detailed research and study can result.

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Introduction

Most literature describes acid preparation of vertebrate fossils without focus on specimen size. In general the material described or illustrated is just a few kilos of combined fossil and matrix (Toombs and Rixon 1959; Rutzky *et al.* 1994; but see Lindsay 1987: Figs 1, 2). An exception is Toombs and Rixon (1959, p.307), who point out that "larger specimens, such as ichthyosaur skulls and associated skeletons of all sizes, present other problems", and go on to describe how the weight of a specimen can damage newly exposed, delicate structures. The Fundación Colombiana de Geobiología (FCG) has undertaken acid preparation of large vertebrate fossils, and here we would like to outline our experience of preparing specimens that can exceed several hundred kilos of initial matrix and fossil bone, and compare this to preparation of specimens of smaller size.

Our results show that, with larger fossil specimens, the chemical properties of the matrix and the fossil bone are highly heterogeneous. This affects a range of chemical preparation parameters, such as the choice of acid(s), as well as the concentration and periodicity of immersions. The volume of matrix; its chemical characteristics; weight, volume and number of fragments; and number of acid bath cycles all affect the preparation in time, and volume of acid consumed. In this paper we wish to share our experience, by citing specimens ranging in mass from small (just a few kilos) to large (weighing several 100 kg), and hence spanning more than two orders of magnitude in weight.

Preparation

The Fundación Colombiana de Geobiología began acid preparation of large marine vertebrates from the Cretaceous sediments of Colombia. The specific specimens referred to here are: the cranium of a small testudine (FCG-CBP40; Figure 1), whose gross weight was 2.3 kg; a large plesiosaurian (FCG-CBP3; Figure 2), whose gross weight (the sum of all the fragments making up the specimen) was 409 kg; and a very large, near complete pliosaurid skeleton (FCG-CBP4; Figure 3), whose gross weight was 728 kg. Where pertinent, we also figure material from other prepared specimens.

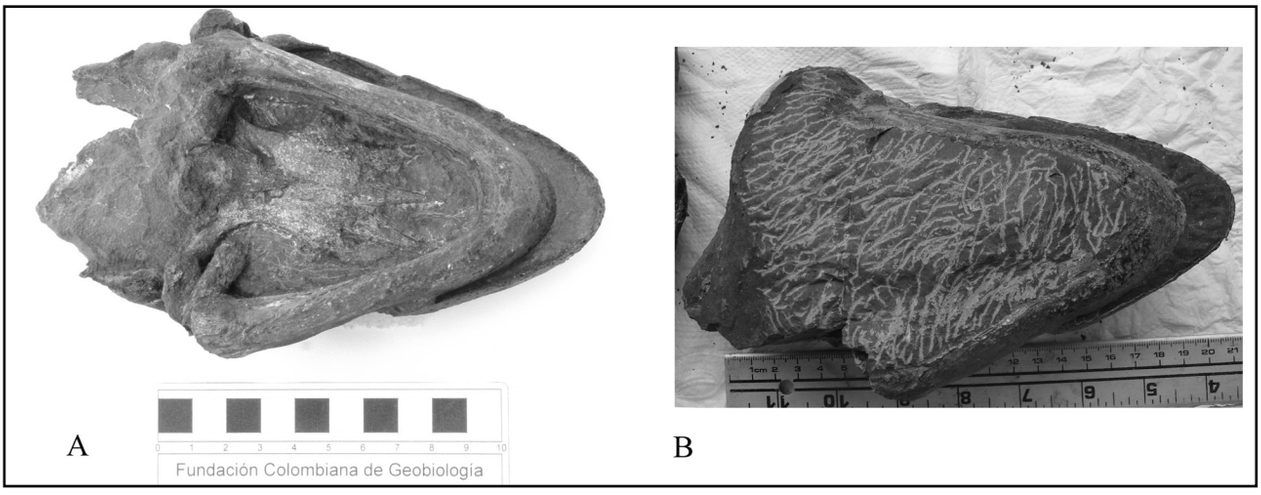

Figure 1. FCG-CBP40, a small testudine (marine turtle) cranium with the lower jaws uppermost and snout to the right. A, prior to acid preparation; B, the partially prepared cranium. Both acid and mechanical preparation were used to assist with matrix removal. Total weight prior to preparation 2.3 kg, scale bars in centimeters.

Figure 2. FCG-CBP3, an almost complete plesiosaur (sauropterygian marine reptile). A as recovered in the field with head in the foreground with snout to the right, and turning away left and towards the rear, B head and neck fully prepared showing exceptional detail of the acid preparation. Weight of specimen prior to preparation was 409 kg, skull 520 mm long, complete specimen 6.22 m.

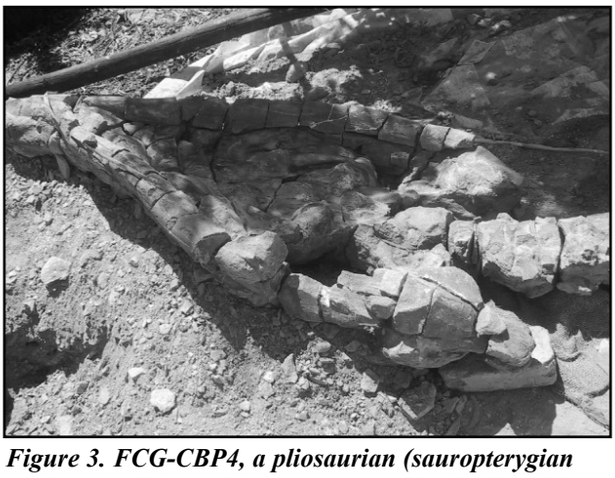

Figure 3. FCG-CBP4, a pliosaurian (sauropterygian marine reptile) cranium as recovered in the field lying with the lower jaws uppermost and snout to the upper left; see also Figure 8. Weight of unprepared specimen 728 kg, length of the cranium 1940 mm.

All specimens were enclosed within a calcareous matrix containing small but varying amounts of non-reactive iron minerals. Acid preparation, combined with standard mechanical preparation, was used to develop the specimens from the matrix. The relevant parameters for optimizing the process of acid preparation of these large vertebrate fossils, compared to smaller specimens, were found to be:

- Size: weight, volume and number of fragments comprising the specimen;
- Choice of acid(s) used;
- Acid bath cycle time and ventilation needs;
- Area of exposed matrix compared to fossil bone exposed;
- Matrix homogeneity;
- Number of protective acid-resistant lacquer coats;
- Managing crevices within the matrix and/or specimen;
- Rate of acid consumption.

Each of these parameters is dealt with in more detail below.

Specimen size

The importance of surface area is a well known parameter relating to solubility in chemical activity (e.g. VP Tyagi 2009, p. 4.7.; Albarède 2003). Hence, the activity of an acid, independent of the acid used, varies with the size and number of fragments that make up the specimen. Smaller fragments have a larger surface area to volume ratio, compared to a single larger specimen of the same mass, and the matrix will be removed more quickly from the smaller specimens compared to larger fragments. The largest element of FCG-CBP4 (Figure 3) weighed 50 kg. Such large specimens require more acid bath cycles to prepare than smaller specimens, or skeletons recovered from the field as numerous loose elements (Figure 4).

The variance in time required to prepare larger versus smaller fragments of a specimen poses challenges to the preparator, as the smaller elements will be fully prepared sooner than the larger ones. Where large and small fragments have contact surfaces, these should be verified after each acid bath cycle to ensure continuing contact. The importance of protecting contact surfaces between fragments cannot be overemphasized in order to ensure a good joint surface after preparation is finished. Indeed it is preferable to overprotect these joint surfaces with multiple coats of acid resistant lacquer, than to risk acid attack and damage to these surfaces. Excess protective coats can then be removed after preparation is finished. These well-preserved fracture and contact points are extremely useful for reconstructing original complex morphology, and for exposing internal anatomical structures, when study of the specimen begins.

A second area of importance to chemical activity is the effect of temperature (e.g. Fisher and Arnold 1999). Acid bath temperature can be expected to have an affect on acid preparation activity; however, in our laboratory temperature is relatively stable, between 18-20°C. We therefore did not focus on this area of study. Nonetheless, acid bath temperature may need to be controlled if ambient temperature varies by many degrees during the course of one, or a series of, cycles. Higher temperatures will provide faster removal of matrix, but they will also increase the fumes produced by the acid bath, and potentially cause more rapid damage to the specimen being cleaned.

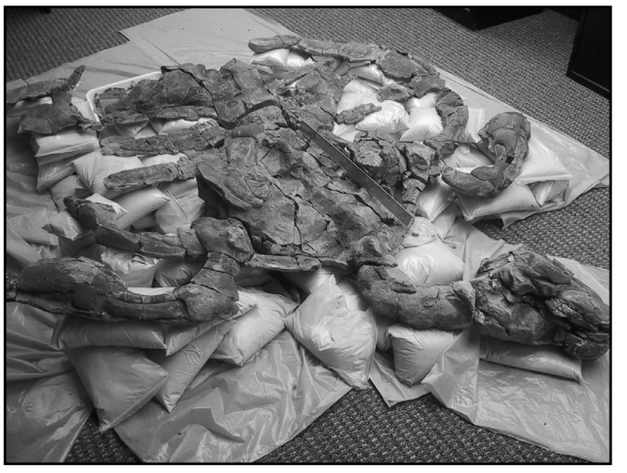

Figure 4. FCG-CBP1, an almost complete skeleton of a large testudine (marine turtle) with head to lower right. The specimen was recovered in many pieces and this fragmentation simplifies acid preparation, however, the junctions between contiguous elements must be extremely well protected from acid attack during preparation to ensure close fit of adjacent fragments. Scale bar 300 mm.

Choice of acid(s)

Acid preparation is carried out with weak acids, generally organic, which disassociate (ionise) incompletely, rather than strong acids, generally inorganic or mineral acids, which essentially disassociate completely. Weak acids are used in order to limit acid activity and potential damage to fossil material (Lindsay 1987); in addition a phosphate buffer is added to the solution to limit acid damage to the calcium phosphate of the fossil bone. Acetic and formic acids, both weak organic acids, are the most commonly used in calcareous fossil preparation (Lindsay, 1987), with acetic preferred in Europe, largely due to the slightly greater health and safety risks associated with using formic acid, and formic in the US because of the more rapid reaction times. Of the two acids, formic is the stronger weak acid, as it has a greater disassociation constant (K_a) thereby providing a greater number of H+ ions to the solution; acetic acid has a lower K_a than formic acid and can therefore be considered a relatively weaker weak organic acid. However, the Fundación Colombiana de Geobiología has also used sulfamic (US English) or sulphamic (UK English) acid (chemical formula H_3NSO_3) which is a somewhat stronger weak organic acid than either formic or acetic acids. Sufamic acid is a colourless crystalline solid with long-term stability during storage.

When the area of exposed fossil is small relative to the volume of matrix, a stronger weak acid is prefer-

able, as it requires fewer, shorter acid immersions (but see Jeppson *et al.* 1985 and Baars 2009 for comments on acids and style of preservation). These more rapid cycles limit the potential for acid to penetrate the specimen and can thus cause unseen damage. If the specimen is already well exposed, or when preparation has advanced exposing more fossil material, a decision can be made as whether to continue with the stronger weak acid or whether to finish the preparation with a weaker weak acid, such as formic or acetic. We have found that a combination of acids produces the best results.

Acid bath cycles and ventilation

When preparing large specimens, results were consistently better using the stronger weak acids to speed up initial matrix removal. The lower dissociation constants of the weak organic acids, compared to mineral acids, allow them to be used at low concentrations (we have standardized sulfamic acid concentration to 2% weight by volume) permitting longer immersion cycles (of up to 8 hours) without causing damage to the fossil material. Historically much greater weak organic acid concentrations have been utilised, starting at up to 33% acetic acid, later reduced to 10-15% where much care is required (Rutzki *et al.* 1994). At the lower concentrations the weaker acetic acid H+ ions are quickly consumed, so that for the most part the acid activity finishes within about 2 hours. Hence the number of acid bath-wash-dry-protect cycles more than doubles compared to stronger acids. However, stronger acids can last many more hours before losing their activity. Due to the longer acid bath cycle times that stronger acids allow at low concentrations, it is important to inspect the cleaning process at least every two hours to assess progress as new fossil is exposed. This will minimise potential damage to the specimen being prepared.

Specimens consisting of many fragments benefit from multiple acid bath tanks being used in parallel for each cycle. This requires more physical space in the preparation laboratory, and a more sophisticated ventilation system. The Fundación Colombiana de Geobiología opted for a significant change in the acid used, incorporating the use of a weak organic acid that does not produce toxic or corrosive fumes; sulfamic acid (whose use is currently being described in another paper to be published elsewhere). The use of sulfamic acid simplifies ventilation to just the CO_2 produced from the activity of the acid on the calcareous matrix. During acid bath cycles, it is important to have a firm spongy material (such as Plastazote foam) to support the fragments being prepared, as direct contact with the bottom or sides of the container may cause damage to the integrity of the fossil or protective films caused by the weight of the specimen (Rutzki *et al.* 1994).

Area of exposed matrix and fossil

The greater the area of matrix exposed on the specimen, the greater the benefit of using a stronger acid to remove the matrix more rapidly with fewer acid immersion cycles. If, on the exposed surfaces, fossil material predominates, starting with a weaker acid is generally a better option to avoid unnecessary stress or damage to the fossil material. However, if matrix dominates then a stronger weak acid will remove more of the matrix more rapidly than a weaker acid. The issue is then one of acid strength and acid speed of action (and hence preparation time) versus potential damage to the specimen, which can be minimised by careful observation whilst the specimen is in the acid and judicious washing with fresh water upon completion.

Matrix homogeneity

Large specimens that may extend over many metres in length, and with varying width and height, are unlikely to be surrounded by homogenous matrix. Hence, matrix heterogeneity is quite usual, and for the Fundación Colombiana de Geobiología material containing ferric (Fe 3+) iron in the matrix is one of the most difficult and intractable problems to deal with. A strategy of mixed acid and mechanical preparation has proven indispensable (Figure 5). Trials on small, 5-10 g, fragments of matrix will quickly indicate where the acid will work best, or where more

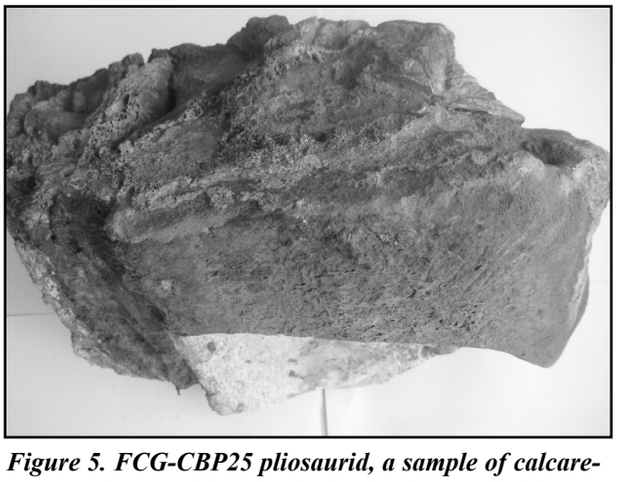

Figure 5. FCG-CBP25 pliosaurid, a sample of calcareous matrix containing ferric (Fe 3+) iron, which is not susceptible to acid attack. This must be removed mechanically for acid preparation to continue. This is a chip of matrix ws used to test the efficiency of the acid on the matrix; field of view approximately 150 mm

Figure 6. FCG-CBP4, the snout of a large pliosaurian with snout to lower left. A, specimen 'overprotected' with several thin layers of B72 polyvinylacrylate to impede acid attack on the bone; B, the same specimen, but inverted, with preparation completed and the excess B-72 removed, to reveal the fully prepared specimen.

mechanical preparation is required.

Number of coats of acid resistant lacquer

Over the years we have experimented with the polyacrylates Butvar (polyvinyl butyral) and Acryloid (or Paraloid) B-72 as acid resistant lacquers. Eventually we have continued to work only with Acryloid (Paraloid) B-72, as we preferred the integrity of the film after repeated handling and acid cycles. To dissolve the B72 we have minimized the use of acetone as a solvent, largely for health and safety reasons, and are currently using industrial grade ethanol. Acetone continues to be used, but only for the removal of excess B-72 after matrix removal is completed.

The outcome of acid preparation of large specimens, which need to be submitted to many acid cycles, depends on the extent of the protection provided to the gradually exposed bone. Rixon (1976) points out that some failures with both formic and acetic acids have been caused by over coating specimens as they are prepared. We agree that a single thick protective layer does not provide adequate protection, as the acid easily undermines a single layer of acid resistant lacquer. In addition, the physical integrity of a single thick layer can be compromised during manipulation and handling of the specimen. However, several thinner layers of acid resistant lacquer, commencing with a solvent wash to displace remaining water, can effectively protect the fossil material over many acid bath cycles. However, it is better to over protect the specimen and later remove excess layers of polyacrylates once the fossil is completely prepared, than to under protect it.

After initial acid cleaning, consisting of immersion of a fragment for a few minutes without any protective coats (Lindsay 1987), the specimen is washed and allowed to dry. To begin protection of the exposed fossil material, initially immerse or brush on pure solvent (we use ethanol) to displace remaining water in pores or crevices, followed by one or two coats of very dilute polyacrylate (1-5% wbv). This is followed by application of pure solvent (ethanol) brushed on to assist the dilute protective lacquer solution to penetrate the fossil bone. Once dry, applying one or more final coat(s) of a more concentrated polyacrylate solution (5-15% wbv) will provide the final protection; we have used up to four layers on some specimens, gradually increasing the concentration of the applied lacquer. After each acid cycle, the procedure is repeated to protect the newly exposed bone, and if necessary to reinforce the protection of previously exposed bone. With the preparation completed, the excess polyacrylate is removed with acetone (Figure 6).

Management of crevices

Rixon (1976) indicates that sometimes it is not advantageous to fill the cracks and crevices in the bone until acid preparation is almost complete. We agree that on free bone the selective cleaning of the acid can really bring out the details of suture lines, foramina and other features However, we make the case for occluding small cracks and larger crevices not in the fossil material, but rather in the matrix being removed. Not managing these crevices in the matrix will allow the acid to penetrate deep into the material being prepared and potentially damage the fossil long before the matrix encasing the material has been removed. After the cracks and crevices have received polyacrylate protection, provided by pouring or injecting it into the crevice, the specimen is allowed to dry. Further filling of the crevices to

restrict entry of the acid solution is attained by physically filling in the remaining spaces. The best material is dental wax (Figure 8); however its cost can be prohibitively expensive in large crevices, so a second best option is Plasticine. Both are substances are reusable in the laboratory following completion of acid preparation, and the initial acid resistant lacquer acts as a separator layer between specimen and filler.

Figure 7. FCG-CBP4, plesiosaur paddle bones, with dental wax being applied to the gaps in matrix and bone; field of view approximately 200 mm.

Acid Consumption

Acid consumption is proportional to the volume of calcareous matrix removed, and during the preparation of three fragments of the snout of FCG-CBP4 (Figures 3 and 7), the volume of acid used varied according to the weight of matrix removed. The unprepared weight of each fragment is shown in Table 1 together with the final weight of each prepared fragment and the mass of matrix removed. From this, it was possible to calculate the percentage mass loss, and to observe that, although all three fragments joined together, the amount of matrix removed from each varied considerably. This difference in the volume of matrix removed indicates a difference in the volume of acid required in their preparation. However, as the volume of matrix to be removed is generally unknown at the outset of acid preparation, the consumption of acid is essentially unpredictable. Hence, it is good practice to have a generous supply of acid available to ensure work does not need to be interrupted, thereby wasting valuable preparation time.

Figure 8. FCG-CBP4, the snout of a large pliosaurian (sauropterygian marine reptile), upper surface of cranium with snout to the right; see also Table 1. A, the unprepared snout showing the anterior three fragments; B, the prepared specimen with the three snout fragments in place at the front of the cranium. Length of cranium 1.94 m.

Fragment number	Initial weight	Final weight	Weight of matrix removed	percentage mass reduction
Cranium 1	11.4	4.1	7.3	64
Cranium 2	8.8	7.6	1.2	14
Cranium 3	14.0	12.6	1.4	10

Table 1. Reduction in mass of three consecutive cranial fragments of FCG-CBP4 (Figures 2, 9) following acid preparation. Specimens were weighed prior to and following acid preparation; all weights in kg.

Conclusions

Acid preparation is an excellent choice for removing large vertebrate fossils embedded in a calcareous matrix, as has already been documented for many smaller specimens (Jeppson *et al.* 1985; Lindsay 1987). The use of sulfamic acid, a stronger weak organic acid, in the initial preparation cycles has distinct advantages; it does not produce toxic fumes which simplifies ventilation and increases the number of acid baths that can be used at once. This stronger weak acid increases initial matrix removal and reduces the number acid bath cycle times, without compromising the quality of preparation.

The use of a mix of acids and mechanical preparation techniques not only optimizes the results of preparation, but is almost essential due to variations in the make-up of the matrix across large specimens. The importance of protecting the often fragile fossil material cannot be overemphasized, especially given the number of acid bath cycles required to prepare large specimens. The parameters followed need to be varied to optimize the process given the nature of dealing with large and complexly shaped specimens. The choice of acid(s) should also be carefully selected in order to avoid undue stress to the fossil material, while achieving the preparation goal in an adequate time frame. This combination of factors has produced excellent results with minimal damage to the specimens being prepared, whilst providing excellent material for subsequent research and display.

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Appendix: Materials

Acetic Acid. 99.5 % USP (United States Pharmacopeia), purchased locally from Quimicos ORBE, can be purchased from a chemical supply house such as SIGMA-ALDRICH Corporation, 3050 Spruce Street, St. Louis, MO 63103, USA.

Formic acid. 85% USP, purchased locally from Quimicos ORBE, can be purchased from a chemical supply house such as SIGMA-ALDRICH Corporation, 3050 Spruce Street, St. Louis, MO 63103, USA.

Sulfamic acid. We used a commercial version of sulfamic acid sold for descaling of boilers, evaporators aboard ships; trade name DESCALEX, Unitor Marine Services (reference 571646) which incorporates a pH indicator to show when the acid is spent. Available in 25 kg cans through Wilhelmsen Ship Service at any port in the world: in New York, Wilhelmsen Ship Service, 210 Edgewater Street, US-10305 Staten Island, N.Y., USA, telephone 718-815-9835. It can also be purchased USP grade from SIGMA-ALDRICH Corporation, 3050 Spruce Street, St. Louis, MO 63103, USA.

Ethanol. Industrial grade 96% (denatured), purchased locally from Quimicos ORBE, can be purchased from a chemical supply house such as SIGMA-ALDRICH Corp., 3050 Spruce Street, St. Louis, MO 63103, USA.

Acryloid (Paraloid) B-72. Purchased from Talas, 568 Broadway, N.Y., N.Y. 10012, telephone (212) 219-0770.

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