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ARTICLE

Rhamphotheca removal by a potassium thioglycolate based solution – a complementary technique for complete cleaning of delicate passerine skulls

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ABSTRACT: The horny sheath known as rhamphotheca covers at least partially four bones of upper maxilla and two from mandible, precluding the observation of their structures and hindering proper osteological studies of the beak. The two main methods currently used for skeleton preparation, viz. the use of dermestid beetles and maceration, can effectively remove the rhamphotheca, however, with possible drawbacks to the material and lasting long to clean it entirely. We developed a new method to remove the rhamphotheca by using a potassium thioglycolate plus calcium hydroxide plus urea based solution (commercially available as chemical depilatory creams), which breaks sulfide bridges of keratin thus softening this structure. Results of our tests with 69 passerine skulls suggest the more efficient proceeding is to apply the cream on bill with intervals of one to two hours, repeating these applications during four to seven hours and then rinsing the material. The remains of rhamphotheca and subjacent dermis can be removed easily with tweezers while the material is still wet. This new method does not damage the material and can be applied in skulls already deposited in osteological collections, besides being faster than the other methods, allowing the removal of the rhamphotheca in less than one day.

KEY-WORDS: chemical depilatory cream, jaw bones, osteological preparation, *Palatum osseum*, horny sheath.

INTRODUCTION

The horny covering of the beak of birds, known as rhamphotheca, is a thick, modified integument, which consists of layers of flattened epidermal cells filled with beta-keratin. These cells also exhibit mineralization, characterized by deposits of calcium salts (*e.g.* calcium phosphate as hydroxyapatite crystals) between the keratin proteins in the cytoplasm, which promote the increase of rhamphotheca strength (Lucas & Stettenheim 1972, Spearman & Hardy 1985, Stettenheim 2000). The rhamphotheca hardness, thickness and flexibility vary among different avian groups and even along the length of a single bill (Lucas & Stettenheim 1972). Subjacent to this external sheath of hard-cornified tissue and separating it from the upper and lower jaw bones, there is a thin and fibrous (Spearman & Hardy 1985) but dense and highly vascularized dermis, that becomes thicker near the tip of the bill (Van Hemert *et al.* 2011). The limits between this dermal tissue and the underlying periosteum, in some cases, are not clearly distinguishable (Van Hemert *et al.* 2011).

The rhamphotheca is composed by two subunits, the rhinotheca and the gnathotheca, which cover both inside and outside surface of most bones forming the upper and the lower maxillae, respectively (Van Hemert *et al.* 2011). The rhinotheca hides completely the *Os premaxillare* and the rostral portions of the *Ossa nasale, maxillare et palatinum*, while the gnathotheca occults at least the rostral halves of the *Ossa dentale et spleniale*, totalizing six bones of impaired visualization (Jollie 1957, A. G. *pers. obs.*). Accordingly, the presence of an intact rhamphotheca on bird skull has always been a limiting factor for osteological studies comprising any of these bones by precluding the complete observation of their structures such as grooves, foramina and processes (*e.g.* Bock 1960).

The two main methods currently used in osteological collections for cleaning skeletons – bacterial maceration and use of dermestid beetles (Dermestidae: *Dermestes* spp.), the latter considered more efficient and effective (Williams & Rogers 1989) and preferred for the preparation of articulate skulls (Alvarenga 1992)

– can effectively remove the rhamphotheca, however with possible drawbacks to the material: maceration techniques, based on the rotting process of tissues of the carcass immersed in water by bacterial action (Hamon 1964), do separate the horny sheath from the subjacent bones (Woolfenden 1970), but produce completely disarticulated skeletons and cannot be used in immature birds (Hamon 1964); during the skeleton preparation at dermestid colonies, which clean the bones while feeding on the soft tissues of dry carcasses (Sommer & Anderson 1974), the larvae are capable to eat and remove the rhamphotheca but, through their random action, commonly attack and destroy bones before consuming the corneous covering (A. G. *pers. obs.*). The negative effects of both methods – promoted by the prolonged exposure often necessary to remove the rhamphotheca completely – are even worse to small and fragile skulls, such as those found in Passeriformes. In addition, both procedures take a long time to remove the entire corneous sheath, and in the case of using dermestids, this time can be quite unpredictable (A. G. *pers. obs.*).

Another limitation to the use of these methods to remove rhamphotheca is that they can hardly be applied to skulls of osteological collections, already cleaned but still retaining this horny sheath. The risks and drawbacks of these methods would affect more severely this kind of material, even more fragile with resected membranes of connective tissue and lacking soft tissues to protect it: dermestid larvae could cause a more intense damage to already exposed delicate bones and maceration would promote a complete disarticulation of palatine bones and other non-fused structures before the time needed to remove the rhamphotheca (A. G. *pers. obs.*).

We developed a complementary method to remove the rhamphotheca of skulls already cleaned by the use of dermestid beetles. The new technique is based on the action of a solution containing potassium thioglycolate, calcium hydroxide and urea – commercially available as chemical depilatory creams for hair removal –, which softens and permeabilizes quickly and efficiently the entire rhamphotheca. This product does not damage even small and delicate skulls (*e.g.* passerines) and can be applied to old skeletal material deposited in osteological collections, besides permitting the cleaning of many skulls at the same time.

MATERIAL AND METHODS

We used in our tests 69 passerine skulls (already cleaned of soft tissues by dermestid colonies, but with the rhamphotheca still intact) deposited at the collection of the Laboratório de Ornitologia of the Universidade Federal do Rio de Janeiro (Appendix). These skulls present a great range of total size and beak length, varying respectively from 26.00 mm (*Xenops rutilans*) to 76.00 mm (*Xiphocolaptes albicollis*) and from 11.40 mm (*Xenops rutilans*) to 50.00 mm (*Xiphocolaptes albicollis*). Their time in the collection also varies, being the oldest skulls prepared in 1995 and the newest ones, in the same week of our tests.

The substance formula used in our tests, found as a chemical depilatory product in the form of a white thick cream, presents three key active ingredients: potassium thioglycolate – $C_2H_4O_2S.K$, at a concentration of 9.5% to 10% –, calcium hydroxide – $Ca(OH)_2$, at a concentration of 3.5% to 4% – and urea 46% – CH_4N_2O, at a concentration of 7% to 9% (Reckitt Benckiser 2009, Abrutyn 2011). Potassium thioglycolate (just as thioglycolic acid and other alkaline salts derived from it) acts breaking the disulfide bonds (S-S) formed between cysteine units of keratin molecules (Abrutyn 2011) present in the rhamphotheca, destabilizing its structure, softening it and making it permeable. This reaction requires a high pH medium to occur, which is guaranteed by calcium hydroxide, a strong alkaline base (Abrutyn 2011). The substance used in this study presents pH between 12.2 and 12.5 (Reckitt Benckiser 2009). The speed of keratin disintegration is increased by the action of urea, which helps the fast penetration of the cleavage agents by swelling the corneous tissue (Abrutyn 2011).

In all tests we used the same chemical depilatory cream for hair removal (VEET ® hair removal cream, sensitive skin), however, other cosmetic products containing similar active ingredients (thioglycolic acid/salt plus hydroxide base plus urea) would be also efficient for this purpose. Due the high alkalinity and keratin destruction promoted by these products, it is recommended to wear nitrile gloves while handling them for a long time (Reckitt Benckiser 2009).

The tested method consisted of successive applications of the cream at the entire surface of rhinotheca followed by scraping off the cream with a small metallic spatula. This alternation between application and scraping was repeated until the corneous sheath became thinner, spongy and opaque, and its color lightened. Then, we rinsed the skulls under running tap water while scraping gently the rhamphotheca. The remaining of rhamphotheca and the entire subjacent dermis were removed with tweezers while the material was still wet. Before leaving the material to dry, we immersed the skulls in ethanol 90% for 30 minutes in order to accelerate drying and thereby prevent the bad smell of the material and weakening of the connective membranes.

It is possible to interrupt the process at any stage, continuing from the stop point days later. It is necessary, however, to wash the cream from the skull and dry it by immersion in ethanol before storage to avoid damages to the material. Before removing the rhamphotheca remains and dermis after drying, it is recommended to

re-hydrate the beak by involving it with wet cotton for two hours.

To determine the optimal interval between applications to obtain the most efficient action of the cream, we initially submitted six skulls to three different protocols, varying the frequency of application of the product: the arbitrary protocols established an interval between cream applications of every half hour, every one to two hours or every three hours. Each pair used in this test involved skulls of *Manacus manacus* and *Passer domesticus*, thus representing both delicate and sturdy beaks. The effective degradation of the rhamphotheca was monitored after each scrape by observing the aspect of the beak, the presence/absence of soft fragments of rhamphotheca mixed with the removed cream and, mainly, the color of the cream scraped from the beak, which acquires tones ranging from light to dark brown – almost black – depending on the amount of keratin and melanin dissolved in the cream, thus staining it.

After determining the most efficient protocol for the cream applications, we adopted it for the large-scale test of the method, applying it to the remainder of the material. During this test, we recorded the number of cream applications and the total time necessary to remove the rhamphotheca of each specimen.

RESULTS

The new method has proven widely effective for removal of the rhamphotheca, promoting a massive flaking and softening of this corneous sheath, thus allowing an easy manual cleaning of its remains. No damage to any skull was observed and, given that the time of contact between skulls and water was very short and the material was not submitted to any mechanical stress (neither during scraping nor during the manual removal of rhamphotheca fragments and dermis), no bones were disarticulated during the process, even in the case of the smaller skulls.

The cream action was more efficient with the intervals of one to two hours between applications. The other tested time intervals presented weaker results or some inconveniences for the efficacy of the method: periods of half hour seemed too short for a satisfactory action of the cream, since the original features of the beak (color, texture and polish) and of the removed cream (color and consistency) remained almost unchanged after the applications; periods of three hours resulted in the dehydration of the cream, hampering the removal of it with the rhamphotheca and affecting the speed of the reaction, which performance was very similar to the observed at intervals of one to two hours (considering the aspects of the beak and of the removed cream after scraping).

The total time needed for the chemical degradation of rhamphotheca varied from four to 30 hours, and the number of applications of the cream ranged from two to 15. Despite this apparent great variation, the majority of the specimens (54 skulls) were completely prepared in the period of four to seven hours, with two to six applications, and for the other skulls, except two, the process lasted from eight to 12 hours. We did not find any association between the total time/number of applications needed to remove the rhamphotheca and the length of the beak or the age of the skull in the collection.

Thereby, we propose as the best protocol for this method: 1-Apply a thick layer of depilatory cream on all surface of rhamphotheca, 2- Wait an interval of one to two hours and then scrape the cream with loose fragments of the rhamphotheca, 3- Repeat the application and the scraping (steps 1 and 2) until the rhamphotheca become thinner, spongy and opaque with a lighter color (on average after two to six applications), 4- Rinse the skull in running water, continuing to scrape the remainder of the rhamphotheca, 5- Remove with tweezers the remains of the rhamphotheca and of the dermis while the skull is wet, in a stereoscope microscope if necessary, 6- Immerse the skull in ethanol 90% for 30 minutes and then, wait the material dry completely.

DISCUSSION

Two specimens curiously required an exceptionally long time of about 30 hours and 15 cream applications for the removal process to be completed. Both skulls, representing *Cymbilaimus lineatus* and *Hypoedaleus guttatus*, were not notably large, nor possessed the longer beaks nor were specially new or old in the collection when compared with the other skulls used in this test. Perhaps this resistance to the method might be related to their characteristic beak, very robust and heavy (Zimmer & Isler 2003). Probably this kind of bill presents a thicker rhamphotheca contributing to this tough structure, since the strength of the whole beak depends on the arrangement of bones and the horny sheath (Spearman & Hardy 1985).

Despite this delay, the new method is still the fastest for removing the rhamphotheca when compared to maceration and the use of dermestid colonies. Besides, this technique makes the preparation of osteological material safer, since it is not necessary to expose skulls any longer to the risk of damage by dermestid beetles and disarticulation by maceration just to remove the rhamphotheca, which can be easily removed at any time by posterior treatment with depilatory cream as presented herein.

The use of this method might be extended to other avian groups, such as hummingbirds (Trochilidae), swifts

(Apodidae), and some small woodpeckers (Picidae), contributing for a better osteological preparation of delicate skulls. Future studies concerning the applicability and efficacy of this technique to larger bird skulls with more massive bills would be interesting, considering the huge diversity of beak morphology in Aves.

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APPENDIX:

Specimens used to test the efficacy of rhamphotheca removal by potassium glycolate based solution (Classification according to Remsen *et al.* 2012):

Order PASSERIFORMES

Suborder TYRANNI

Family THAMNOPHILIDAE

Cymbilaimus lineatus (Leach 1814): UFRJ 1095.

Hypoedaleus guttatus (Vieillot 1816): UFRJ 1083.

Thamnophilus ambiguus Swainson 1825: UFRJ 0294, UFRJ 0295, UFRJ 0296.

Thamnophilus palliatus (Lichtenstein 1823): UFRJ 0289.

Myrmotherula axillaris (Vieillot 1817): UFRJ 0290, UFRJ 0293, UFRJ 0386.

Myrmotherula gularis (Spix 1825): UFRJ 0484.

Myrmorchilus strigilatus (Wied 1831): UFRJ 0690.

Herpsilochmus rufimarginatus (Temminck 1822): UFRJ 0297, UFRJ 0378.

*Herpsilochmus sellowi (*Whitney & Pacheco 2000)*:* UFRJ 0380, UFRJ 0381.

Formicivora iheringi Hellmayr 1909: UFRJ 0325, UFRJ 0327, UFRJ 0328.

Drymophila squamata (Lichtenstein 1823): UFRJ 0372, UFRJ 0375, UFRJ 0530, UFRJ 0766, UFRJ 0767.

Drymophila ferruginea (Temminck 1822): UFRJ 0493, UFRJ 0625.

Pyriglena leucoptera (Vieillot 1818): UFRJ 0251, UFRJ 0254, UFRJ 0506, UFRJ 0534.

Family CONOPOPHAGIDAE

Conopophaga melanops (Vieillot 1818): UFRJ 0267, UFRJ 0876.

Family RHINOCRYPTIDAE

Merulaxis ater Lesson 1830: UFRJ 0872, UFRJ 0877.

Family FORMICARIIDAE

Formicarius colma Boddaert 1783: UFRJ 0397, UFRJ 0398, UFRJ 1239.

Chamaeza ruficauda Cabanis & Heine 1859: UFRJ 404.

Family FURNARIIDAE

Sclerurus scansor (Ménétriès 1835): UFRJ 1015, UFRJ 1019.

Xenops rutilans Temminck 1821: UFRJ 0490, UFRJ 1041.

Furnarius rufus (Gmelin 1788): UFRJ 0763, UFRJ 1081.

Lochmias nematura (Lichtenstein 1823): UFRJ 0867.

Anabazenops fuscus (Vieillot 1816): UFRJ 1034, UFRJ 1042.

*Philydor atricapillus (*Wied 1821)*:* UFRJ 0496, UFRJ 0978.

Automolus leucophthalmus (Wied 1821): UFRJ 0979, UFRJ 1064.

*Phacellodomus erythrophthalmus (*Wied 1821): UFRJ 0399, UFRJ 0400.

Synallaxis albilora Pelzeln 1856: UFRJ 0537.

Sittasomus griseicapillus (Vieillot 1818): UFRJ 1089, UFRJ 1090, UFRJ 1098.

Dendrocincla turdina (Lichtenstein 1820): UFRJ 0410.

Dendrocolaptes platyrostris Spix 1825: UFRJ 1022, UFRJ 1023.

Xiphocolaptes albicollis (Vieillot 1818): UFRJ 1027, UFRJ 1094.

Xiphorhynchus fuscus (Vieillot 1818): UFRJ 0508, UFRJ 1092.

Family PIPRIDAE

Manacus manacus (Linnaeus 1766): UFRJ 504, UFRJ 523, UFRJ 525.

Suborder PASSERES

Family PASSERIDAE

Passer domesticus (Linnaeus 1758): UFRJ 613, UFRJ 694, UFRJ 696.