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Advances in insect preparation: bleaching, clearing and relaxing ants (Hymenoptera: Formicidae)

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Abstract

Myrmecologists use a variety of methods for clearing (macerating) and relaxing ants. Bleaching, however, has virtually never been applied in morphological studies. Here we describe a combination of a common bleaching treatment of insect cuticle with hydrogen peroxide and a subsequent clearing of adhering soft tissues with either lactic acid or proteolytic enzymes. This technique allows viewing of the internal morphology of ants without dissection. The resulting glassy specimens reveal valuable morphological characters and may be used as three-dimensional morphological maps to guide the dissection of additional specimens. Bleached specimens are thus particularly useful as teaching material. Positive side effects of the treatment are the extension of (a) retracted mouthparts, (b) sting apparatus and (c) armatures of the male genitalia. An underwater preparation procedure with subsequent fan drying is also described for relaxing specimens preserved in absolute ethanol. Based on tests of relaxing methods, a much less harmful but equally effective modification of the carcinogenic Barber's fluid is described for relaxing and cleaning purposes of pinned or card-point mounted specimens.

Key words: Hydrogen peroxide, proteolytic enzymes, lactic acid, Barber's fluid, ethanol preservation, transparent ants.

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Introduction

The continuing biodiversity crisis urgently demands the assessment of our planet's biological diversity and this requires the best possible preservation of specimens for current and future studies (KING & PORTER 2004). The diverse nature of invertebrate groups and the various entomological disciplines require a broad array of preparatory techniques to assure specimen preservation (MARTIN 1977, SCHAUFF 1986, UPTON 1991, UPTON 1994, PIECHOCKI & HÄNDEL 1996, UYS & URBAN 1996, EGUCHI 2002, LATTKE 2000, MILLAR & al. 2000). Species-specific diagnostic features and other morphological characteristics of insect specimens often have to be revealed by preparatory treatments like bleaching, clearing and relaxing. Complex structures like mouthparts, sting apparatus and male genitalia are mostly retracted and often polluted with solidified body fluids, dirt or even glue in the case of card-point mounted specimens. Legs and antennae frequently conceal other body parts that are important for identification. Dark colours can complicate the analysis of the degree of cuticle sclerotisation and prevent viewing of internal structures without dissection. Thus, fully transparent but intact specimens are very desirable. Additionally, glassy specimens can provide three-dimensional morphological maps for dissection of other similar specimens and are valuable for teaching material in entomology courses. BAUERMEISTER (1959) published a time-consuming method to produce transparent molluscs based on the refraction indices of tissues but this approach seems impractical for daily use with insects.

This study on bleaching ants was inspired by our colleague, Dr. Timo Moritz (pers. comm.), who transforms alcohol preserved and formalin fixated fishes into entirely transparent specimens based on the work of DINGERKUS & UHLER (1977). We present a simple, effective technique for routine use based on a combination of hydrogen peroxide bleaching with clearing procedures. We describe how retracted mouthparts, sting apparatus and male genitalia can be extended in bleached specimens. We refine an underwater preparation procedure with subsequent fan drying for relaxing and a better handling of ants intended for card-point mounting. Finally, useful chemical solutions like Barber's fluid (VALENTINE 1942) for cleaning and relaxing stiff and brittle specimens in dry collections have either frequently fallen into oblivion or are unacceptable due to their toxicity. We offer a modified recipe for Barber's fluid which is a less harmful version of the carcinogenic original.

Material

We chose the ponerine ant *Pachycondyla analis* (LATREILLE, 1802) (Hymenoptera: Formicidae: Ponerinae) as a model organism (see Fig. 1). The heavily sclerotised, dark-coloured, polymorphic workers range from approximately 9 mm (minor worker) to 19 mm (major worker) in total body length. The species has winged males and pupae with dark brown silky cocoons. All specimens were collected by the first author approximately 4 km west of the Blue Nile River, Sudan (12° 45' 34" N, 34° 06' 45" E; 444 m a.s.l.), July 2004. Immediately after collection all specimens

were killed with and preserved in absolute ethanol (p.A., min. 99.8 volume percent ethanol) until used for our experiments. The specimens did not show any indication of leaching or bleaching due to their long storage in their preservative. All recipes and details of the chemicals are given in Box 1. Photographs were taken with the single-lens reflex digital camera Nikon D70 equipped with a "1:2.8/105 mm AF Micro Nikkor" macro lens. A single fluorescent light bulb (E27, 15W) served as light source.

Methods and Results

Bleaching with hydrogen peroxide

Depending on the internal structures which are to be revealed bleaching with hydrogen peroxide (for a view on soft tissues like muscles and tendons) or bleaching in combination with clearing (for a view on cuticular structures) had to be chosen. Based on comparative tests using hydrogen peroxide with concentrations from 10 to 35%, the best results were obtained with a 35% concentration. Solutions stronger than this were increasingly hazardous because of their strong oxidising properties and should be avoided.

Major workers, males and pupae within their cocoons were taken from the absolute ethanol preservative and individually submerged in excavated glass blocks containing the 35% hydrogen peroxide. The blocks were covered with small glass plates to prevent desiccation and stored in a bright environment at room temperature. Depending on the colour and the degree of sclerotisation of the cuticle this treatment required between a few hours and 13 days. The specimens brightened within 24 hours. After 48 hours they were noticeably transparent except for the more sclerotised parts of the cuticle such as the head and the alitrunk (see Fig. 2). In an early stage of bleaching when the cuticle already was transparent but soft tissues were not yet macerated to pulp, the internal tissues adjacent to the inner side of the cuticle could be viewed (e.g., muscles and tendons within the legs). Finally the tissue pulp spread non-uniformly within the body.

The petiole and the gaster developed transparency. The cocoons lightened from dark brown to light brown and the commonly adhering soil particles detached. The cocoons were not distended by nascent oxygen due to their porosity. After one to two weeks the cuticle of *P. analis* workers and males appeared completely transparent and glassy. Oxygen bubbles were mainly present in the alitrunk, the coxae and the gaster. The wings of the males lost part of their lifelike shape by curling up at their tips. The cocoons of the pupae entirely dissolved leaving the bare pupae behind.

As soon as the cuticle became transparent and offered an unhindered view on inner structures, the specimens were taken out of the hydrogen peroxide bath and thoroughly rinsed with water or absolute ethanol depending on the next treatment. For subsequent clearing with proteolytic enzymes or lactic acid, water turned out to be superior but for preservation alcohol was used. The results of the bleaching procedure depended on the light intensity, the duration of bleaching and the concentration of hydrogen peroxide. Bleaching in a bright environment was faster than in the dark. The longer the immersion and the higher the concentration of hydrogen peroxide, the more light-coloured was the cuticle of the specimens. A side effect of bleaching was a decrease in the thickness and stability of the cuticles. Soft-bodied specimens like workers or males freshly emerged from their pupae tended to be partially disintegrated if bleached for too long. Specimens left for three weeks in hydrogen peroxide became too soft for pinning or card-pointing.

Fig. 1: *Pachycondyla analis*, intermediate worker in absolute ethanol before bleaching and clearing.

Fig. 2: *Pachycondyla analis*, intermediate worker after 3 days of bleaching with 35% H_2O_2.

Bleaching in combination with clearing

Bleaching specimens in a 35% hydrogen peroxide solution lead to transparent cuticles and to pulped, non-uniformly distributed internal tissue fluffs. Specimens were subsequently cleared by treatment with lactic acid or the proteolytic enzyme pepsin as follows. First, the specimens were bleached as above just until they reached transparency for an unhindered view of targeted cuticle regions without too much thinning of the cuticle. Second, the specimens were thoroughly washed in water to eliminate remaining hydrogen peroxide. The samples then were transferred into excavated glass blocks filled with either lactic acid or a pepsin-solution (see Box 1). The blocks were covered with glass plates and stored in a dark place at room temperature for up to 13 days. During this period, it was occasionally necessary to renew the pepsin-solution once or twice when evaporation occurred. The appearance of flocculation inside specimens indicated an incomplete diges-

Box 1: Recipes.

Barber's fluid after VALENTINE (1942), in order of mixture!

Original recipe (carcinogenic)		**our modified recipe,** pH-value: 4	
265 parts	95% ethanol	51 ml	100% ethanol
35 parts	benzene	7 ml	acetone (pure)
95 parts	ethyl acetate	19 ml	ethyl acetate (pure)
245 parts	water (aqua dest.)	51 ml	water (aqua dest.)

Lactic acid (IUPAC systematic name: 2-hydroxypropanoic acid)

Pure quality, pH value: 0.8

Pepsin-solution after KLESS (1986), pH value: 1.6

2.5 g	powdered pepsin
2.5 ml	concentrated hydrochloric acid
100 ml	water (aqua dest.)

Trypsin-solution after TAYLOR & VAN DYKE (1985), pH value: 9

2 g	powdered trypsin (= pancreatin, pancreatic protease, purified trypsin)
75 ml	filtered saturated sodium borate solution (sodium tetraborate, $Na_2B_40_7 \cdot 10H_2O$)
25 ml	water (aqua dest.)

tion. When maceration had entirely cleared all soft tissues, specimens were taken out of the blocks, rinsed thoroughly with water and transferred into absolute ethanol or glycerine for preservation. Oxygen bubbles emerging during the bleaching procedure were eliminated by storing the specimens in absolute ethanol.

Clearing with either lactic acid or proteolytic enzymes (pepsin) initially produced similar results. The remaining tissue fluffs were completely digested and a fully transparent, glassy ant was obtained (see Fig. 3). In a few cases, the use of pepsin created poor specimens when they were transferred too quickly into absolute ethanol. The degradation products of incompletely digested soft tissues (formerly soluble in water) precipitated throughout the entire specimen. The appearance changed immediately from transparent to translucent. In general, lactic acid and pepsin did not weaken the cuticle in contrast to long immersion in 35% hydrogen peroxide solution. Enzymatic clearing alone also proved to be an efficient means of extracting large series of ant pupae out of their cocoons without damaging their fragile structures. A reverse chronological order of treatments, i.e., clearing with subsequent bleaching, proved suboptimal as this led to specimens being translucent rather than really transparent.

Bleaching for extending retracted mouthparts, sting apparatus and male genitalia

The specimens were treated as described in the previous two sections but the duration of the treatment was reduced to 3 to 5 days. The hydrogen peroxide bath was halted when either the retracted appendages protrude or the organs could be extracted easily with a forceps or a hooked insect pin. In about one third of the *P. analis* specimens, the mouthparts were extended. With the remaining specimens, closed mandibles could usually be opened without force and without breaking the mandibular muscles. Generally the specimens and their appendages were relaxed and ready to be rearranged.

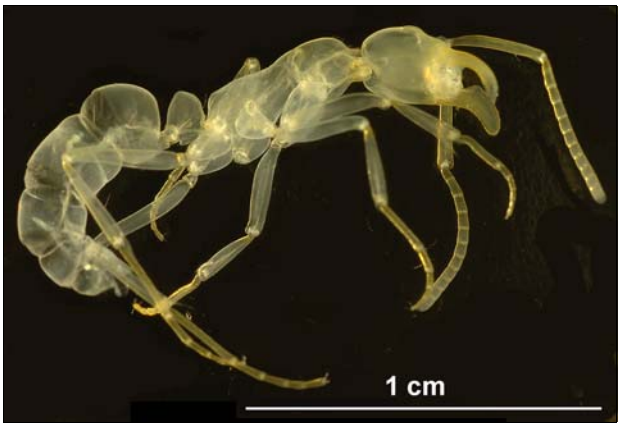

Fig. 3: *Pachycondyla analis*, major worker after 14 days in 35% H_2O_2 and subsequent clearing with lactic acid.

Retracted structures were extended as a side effect of bleaching. This effect resulted from the combined effects of soaking, macerating and pushing, with the latter due to an increasing internal pressure of the nascent oxygen. Structures like mandibles protruded during the procedure or were pulled out easily. The sting apparatus became movable and the male genitalia and entire gaster stretched out of most of the specimens. The boundary of sternites and tergites with the intersegmental membranes were exposed and this allowed precise dissections free from the risk of damage.

Underwater preparation and card-point mounting of specimens preserved in absolute ethanol

For pinning or card-mounting ants that had been preserved in absolute ethanol the specimens were transferred into

Petri dishes containing a layer of paraffin wax, about five millimetres thick and totally submerged in distilled water. After a few minutes of soaking the specimens were carefully turned so that the ventral parts face downward. Without piercing the specimens, lifelike postures of the legs, antennae, wings and the head were fixed with insect pins on the wax layer. Care had also to be taken when manipulating the joints of the funiculus or the tarsi to avoid possible breakage. The hind parts of the wings usually needed special support from additional pins to prevent folding up during subsequent treatments. Occasionally, specimens of the same size, colour, and colony reacted differently.

As soon as the specimens were temporarily fixed, the water within the dishes was emptied and the surface of the ants was rinsed with absolute ethanol for a few seconds. The alcohol was poured out and the position of appendages, especially of the wings, was checked and rearranged if necessary. Blow-drying with a hair drier or a computer fan was then used until the pubescence, pilosity and, particularly, the wings were dried without sticking together thus providing a natural appearance. When the wings folded up the procedure of moistening, smoothing and drying had to be repeated. After blow-drying the surface of the ants, the whole specimens were left to dry naturally in air until their appendages became stiff. Then all insect pins were removed with care and the specimens were mounted on card points in the conventional manner (PIECHOCKI & HÄNDEL 1996). When large specimens required direct pinning this was done before the drying procedure. The method permitted rearrangement of most of the appendages (legs, head, antennae and gaster) except for the wings, the posture of which could not be changed due to the position of thoracic plates at the time of death.

A modified Barber's fluid for cleaning and relaxing of mounted or dry preserved specimens

Barber's fluid (VALENTINE 1942) is used mainly for cleaning and relaxing specimens or their appendages. We modified the original recipe by substituting acetone for the carcinogenic benzene (see Box 1). The ingredients were mixed at room temperature in the order alcohol, acetone, ethyl acetate and finally distilled water. When the liquids did not mix entirely, we followed the advice of OEHLKE (1967) to warm up the fluid using a water bath.

Generally the Barber's fluid was locally applied with a pair of tweezers or a fine hair brush, but we also submerged specimens in the fluid. A few drops sufficed for cleaning card-point mounted specimens polluted with non-water-soluble glue. Within seconds the glue dissolved and the affected body parts could be cleaned. In the same way we used the fluid to remove glued specimens from their card-points. Without exception the glues were successfully removed, even old glue of unknown composition. For relaxing stiff extremities of dried ants, the fluid was also effective. Legs and coxae of card-point mounted specimens that were brushed with the fluid became movable in less than a minute. Femora and tibiae were adjustable as well. Structures like mandibles sometimes needed repeated brushing or even partial submerging in the fluid for similar results. Whenever appendages resisted the slight force of the forceps while testing, the fluid was repeatedly applied to enable further penetration into specimens. Immersion in the fluid also proved effective for relaxing whole specimens formerly preserved in alcohol. Alcohol killed and preserved material was dried under a lamp and slightly moistened with the fluid as done by VALENTINE (1942).

Discussion

Bleaching with hydrogen peroxide

During bleaching, hydrogen peroxide disintegrates into water molecules and free oxygen radicals through autolysis, with the latter causing the bleaching of the colour pigments of the insect cuticle. With a 35% hydrogen peroxide solution we produced a transparent cuticle and offered a unique view onto lightened internal soft tissues previously hidden by cuticle. Since the bleaching solution is water-based, rinsing with water will not distort specimens. We focused our experiments on a heavily sclerotised species. If weakly sclerotised species are to be preserved in alcohol specimens should be treated stepwise with baths of gradually increasing alcohol concentrations as published by PIECHOCKI & HÄNDEL (1996). With bleaching we adopt a less time-consuming method than the procedure based on the refraction indices of tissues suggested by BAUERMEISTER (1959). Bleaching is a widespread method of selectively eliminating pigmentation of body structures for different purposes, such as subsequent staining. It has been used for the bones of vertebrates like fish (TAYLOR 1967), and for invertebrates like molluscs (PIECHOCKI & HÄNDEL 1996). It has also been used for insects (BODE 1975, SCHAUFF 1986, UPTON 1994, MILLAR & al. 2000).

Bleaching in combination with clearing

Glassy specimens consisting solely of cuticle can be achieved by a combination of bleaching the cuticle and totally clearing the inner soft tissues. Prolonged immersion in the hydrogen peroxide solution leads to maceration but in contrast to proteolytic enzymes and lactic acid weakens the cuticle's strength and structure. To avoid too much thinning, bleaching should be stopped as soon as the cuticle becomes sufficiently transparent to view underlying structures. Subsequent clearing of the remaining tissue is best achieved using the proteolytic pepsin devised by KLESS (1986). However, earlier clearing experiments for male genitalia preparations of *Pachycondyla analis* revealed that a trypsin solution (TAYLOR & VAN DYKE 1985) worked as well, if not better (M. Stüben, unpubl.). M. Maruyama (pers. comm., Kyushu University Museum, Japan) uses protease K (a broad-spectrum serine protease) both for DNA extraction and genitalia cleaning in order to finally embed slide-mounted male genitalia in Euparal. The advantage of using proteolytic enzymes is that membranes possessing chitinous structures are left intact unlike those treated with the more customary potassium hydroxide (KOH) solution (KANAAR 1990). Maruyama (pers. comm.) also uses KOH on occasion and confirmed that genitalia of small ant species are sometimes crushed or modified, if not carefully cleared at low temperature and low concentrations of KOH. EGUCHI (2002) presented a technique for clearing ant male genitalia with a consecutive treatment of KOH, lactophenol, aceto-salicylate and finally carbo-xylol in preparation of mounting genitalia parts on a slide glass with Canada balsam.

In entomology, clearing by dissection or maceration is used when the soft inner tissues hinder the study of the hard chitinous parts and membranous cuticle. Maceration

is achieved most commonly by the use of caustic solutions, such as potassium hydroxide or sodium hydroxide solution, lactic acid (MILLAR & al. 2000) or by proteolytic enzymes (PIECHOCKI & HÄNDEL 1996). These approaches allow morphological studies of mouthparts, sting apparatus or the male genitalia armature (HERING 1931, CLAUSEN 1938, GURNEY & al. 1964, WEISE 1970, KANAAR 1990, CUMMING 1992, EGUCHI 2002, EGUCHI & BUI 2007). Relaxation of stiff specimens is another application especially when other relaxing methods fail (KLESS 1986, MENZEL & MOHRIG 1991). SCHAUFF (1986) suggests bleaching insect specimens that remain too dark after maceration.

The production of whole, fully transparent insect specimens enables *in situ* functional and morphological studies of mouthparts, sting apparatus and male genitalia of ants, and probably insects in general, prior to a dissection or in parallel with dissections of other specimens from a serial sample. Thus the investigator can become familiar with the structures which will help in any further anatomical preparation.

Bleaching for extending retracted mouthparts, sting apparatus and male genitalia

One of the benefits of using the bleaching solution was that mouthparts, sting apparatus, and male genitalia became extended. Nascent oxygen generated inside the cavities of the exoskeleton increased the inner pressure. In specimens with intact chitinous membranes, this pressure distends or at least loosens previously retracted structures. The watery solution together with the nascent oxygen bubbles also cleanses those structures from solidified body fluids and dirt. Thus our method is superior to the conventional use of relaxing water chambers that do not benefit from the oxygen pressure. Producing an internal pressure is an alternative method when structures resist relaxing treatments with steam or with our modified Barber's fluid and when clearing the specimen with caustic solutions or a proteolytic enzyme is not practicable.

MCRAE (1987) reported excellent results with a method of soaking adult flies briefly in diethyl-ether, and then removing them to let the vapour pressure extend the proboscis. We tested MCRAE's method on the mandibles of absolute ethanol preserved and air-dried *Pachycondyla analis* specimens but it did not work due to the heavily sclerotised exoskeleton and strong mandibular muscles (M. Stüben, unpubl.).

Underwater preparation and card-point mounting of absolute ethanol preserved specimens

Sampling and storing ants in alcohol has a long tradition in museum collections. For many years, following the advice of VIEHMEYER (1918), concentrations of about 70 to 75% were used to prevent the hardening of ant specimens to keep them in a workable condition for mounting. KING & PORTER (2004) confirmed the common experience that specimens killed and stored in 70% and 85% ethanol are generally flexible and rubbery, with their legs and antennae readily manipulated without damage, but the appendages tend to return to their original position and this hinders mounting. Additionally, specimens stored in 70% alcohol for six months were noticeably swollen, typically with distended pleural and intersegmental membranes on the abdomen. The difference between these suboptimally preserved specimens and well preserved ants is self-evident.

The demands of preparatory techniques have changed. Many collectors are conscious that species which are sampled and preserved have to stand the test of time and have to ensure multi-purpose studies, because there might never be a second chance of re-sampling due to environmental changes and dramatic biodiversity decline. Molecular techniques have increasingly become an integral component of systematic and ecological work on ants. KING & PORTER (2004) recommend killing and storing ants in 95 - 100% ethanol because DNA is best preserved at this type and concentration of alcohol. They further recommend that adult ants should be killed and stored in 95% ethanol because, in addition to ensuring the preservation of DNA, this will produce specimens that are easier to mount compared to overly brittle specimens resulting from higher concentrations or rubbery specimens from lower concentrations.

Despite KING & PORTER's (2004) recommendation, the recent emphasis on molecular techniques has led some myrmecologists to use only absolute ethanol. The unfortunate result of this is that specimens become too hard or brittle for proper curation without additional treatment. Without further treatments (e.g., with relaxing fluids or relaxing chambers) these specimens have limited value for taxonomic identification. A solution is to use short-term water soaking to speed up relaxation (KING & PORTER 2004). This method is effective in softening specimens from absolute ethanol because the alcohol works as a carrier both inside and on the surface of the specimen (PIECHOCKI & HÄNDEL 1996). Following water-softening, specimens can be rinsed with absolute ethanol and blow-dried to prevent the pilosity and pubescence from sticking together.

Our procedure should be accessible and easy to use for amateur and professional entomologists because the components are standard in insect preparation techniques. As a comparison, OEHLKE (1967) treated ichneumonid wasps preserved in 70% ethanol for seconds to minutes with 96% ethanol before drying under a heat lamp to prevent pubescence from sticking together. Blow-drying accelerates this process and gives the most lifelike posture of the mounted or card-point mounted specimens without affecting pubescence and pilosity. This outcome is especially useful for producing specimens for reference collections and entomological exhibitions, where samples should be of the highest quality.

With care taken to avoid shrinking or swelling of the samples, our method can be easily adapted to other concentrations of alcohol. For colour sensitive insect groups we recommend the technique of MCRAE (1987), using a sequence of baths in 80% ethanol, absolute ethanol and finally diethylether to produce mounted specimens, and modifications compiled by PIECHOCKI & HÄNDEL (1996). Sampling and storing ants in Scheerpeltz' fluid (SCHEERPELTZ 1927, 1936) is not recommended for DNA preservation.

A modified Barber's fluid for cleaning and relaxing of mounted or dry preserved specimens

Conventionally dried specimens are most frequently treated with steam in relaxing boxes to bring them back to a workable condition (MARTIN 1977). Using steam with alcohol material, however, is not very efficient. It usually can be applied only to the whole specimen and affects in-

sect wings and pubescence. Most other relaxing methods are no longer in use, despite the continuing need for properly curated specimens. For example, in many ecological studies where passive capture methods (e.g., pitfall trap, light trap, Malaise, fogging) yield huge amounts of insect material, insects are often collected and preserved through direct collection into low-concentration ethanol.

Similarly, large samples of ants are collected and preserved in 60 to 70% ethanol (SEIFERT 1996) or 70 to 80% ethanol because this allows anatomical examinations or pinning of specimens to be done more or less straight from the preservative (PIECHOCKI & HÄNDEL 1996). For that reason, according to B. Seifert (pers. comm.), some collectors prefer Scheerpeltz' fluid (SCHEERPELTZ 1927, 1936).

VALENTINE (1942) was the first to publish the recipe of Barber's fluid which is a very effective and versatile relaxing agent. Its applications include cleaning specimens from old greasy glue, adjustments of extremities during pinning and corrections of specimens in dry collections. The last is increasing in importance because digitising type material has become a common practice (COP-CBD 2006). If specimens are not properly positioned, even technically excellent photographs in exemplary databases like that to be found at www.antweb.org can be of very little value for comparative taxonomic work. Producing high-quality images is costly and labour-intensive. Thus, non-invasive relaxing methods that allow proper adjustment of appendages to expose taxonomically important morphological features, are particularly valuable. The original Barber's fluid contained benzene and this is classified by the World Health Organization as a human carcinogen (FISHBEIN & O'NEILL 1988).

Our modified recipe uses less harmful chemicals but still provides excellent relaxing results. As with the original Barber's fluid (J. Händel, pers. comm.), our specimens became plastic almost instantly and old, greasy specimens were "rejuvenated". PECK (1974) who used the original Barber's fluid as preservative for leiodid beetles (Coleoptera, Leiodidae) reported that it did not harden the tissues of the beetles as alcohol does. We would expect similar results for our alternative recipe.

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Zusammenfassung

Myrmekologen verwenden eine Reihe von Methoden zum mazerierenden Aufhellen und Aufweichen von Ameisen, während das Bleichen für morphologische Studien praktisch unbekannt ist. Wir beschreiben eine Kombination des gebräuchlichen Bleichens von Insektencuticula mit Wasserstoffperoxid mit der Mazeration anhaftender, weicher Gewebe durch Milchsäure oder proteolytische Enzyme. Diese Technik erlaubt einen Einblick in die innere Morphologie der Ameisen ohne Präparation. Die resultierenden gläsernen Ameisen offenbaren wertvolle morphologische Merkmale und können als dreidimensionale morphologische Karte für eine Sektion weiterer Exemplare fungieren oder als Lehrmaterial dienen. Positive Nebeneffekte des Bleichens sind die Erleichterung des Lösens (a) zurückgezogener Mundwerkzeuge, (b) des weiblichen Stachelapparates und (c) der männlichen Genitalarmatur. Eine Methode zur Unterwasserpräparation mit anschließender Lüftertrocknung wird als Präparationsmethode für Ameisen aus absolutem Ethanol beschrieben. Basierend auf Tests von Aufweichmethoden wird eine sehr viel ungefährlichere aber ebenso effektive Variante des als karzinogen eingestuften Barbers Reagenz zum Aufweichen und Reinigen genadelter und geklebter Exemplare vorgestellt.

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