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Conservation of ant material for natural history collections (Hymenoptera: Formicidae)

Alexander Prosche

Internationales Hochschulinstitut - TU Dresden, Markt 23, 02763 Zittau, Germany

E-mail: prosche90@web.de

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Abstract

Correct sampling and preparation of living material build up the heart of all natural history collections. Ideally, the samples outlive their collectors and preparators for centuries. This article gives advice on correct and durable storage of either mounted or ethanol stored material as well as on correct sampling during field work. Furthermore, the advantages and disadvantages of a number of the most common ant preparation techniques for inner and outer body characters are presented, compared, and illustrated. This can either mean a decision between a basket shape arrangement, a preparation according to Wilson, or a preparation in standing position if the whole individual and its morphology is of interest. Dissection methods to obtain information on inner organs involved in pheromonal communication or indicating reproductive status and age are described.

Keywords ants | preparation techniques | *Chthonolasius* | ethanol storage

1. Introduction

The preparation of insect material can be dated back to antique and medieval times when insects – mainly colorful beetles and butterflies – were collected and conserved for appreciation of beauty or artistic design (Harpaz 1973, Morge 1973). In the early seventeenth century, entomology was founded as scientific discipline. Collection became the decisive instrument of documentation and comparison. Famous scientists and collectors, such as Charles Darwin or Alfred Russel Wallace increased the amount of material tremendously and influenced nomenclature until the present times (Berry 2008). These classical materials are conserved until now and have a high value and importance for evolutionists and taxonomists.

In the nineteenth century the collection of insects became popular for the educated middle class, especially the collection of lepidopterans. Later, these collections usually were donated to natural history museums and increased the information about distribution and rarity. Some taxa were more popular than others resulting in nearly continuous information flow for beetles and butterflies and a lack of information for less eye-catching beautiful groups through the decades.

This article deals with the preparation of ants, which belong to the aculeate hymenopterans. These mostly have a strongly sclerotized exoskeleton, which allows preparation without any pretreatment.

Ants can strikingly differ in their respective size (Fig. 1), which requires different modes of preparation. The systematic and scientific collection of ant material started more than 250 years ago when Carl Linnaeus named the first ants and provided their first taxonomic system (Stearn 1959, Larson 1968). Later, taxonomists, such as Carlo Emery (1848–1925) and Auguste-Henri Forel (1848–1931), described many new taxa, substantially extending the number of type specimens (Forel 1901, Emery 1911). There are different methods of preparation, which have advantages and disadvantages regarding stability and visibility of characters. The present study

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aims at a brief summary of the most common techniques for preparation of ant material. This includes the preparation of whole and intact ant bodies as well as the preparation of inner organs, such as wing-musculature or reproductive organs, which provide valuable information about reproductive capabilities.

2. Collecting and general storage

2.1 Collecting of ant material

The preparation of ant material starts with a correct sampling. For the field work a short, stable knife is a proper tool to access small nests in bark, rotten logs, nuts, acorns or snail shells. For opening harder material – such as living wood – a small camping ax is an applicable tool. In order to lift stones partially sunken into soil, a robust, modified screwdriver is most effective, the tip of which is bent rectangularly within the plane of the apical flattening, about 2–3 cm from the tip. Furthermore, a hand shovel is a good tool for work in loose soil substrates (Seifert 2018). Ant material is usually collected using an aspirator to catch several individuals or a whole nest community. In the latter case, information about colony size, number of gynes, number of eggs, larvae, and pupae or the occurrence of social parasites can be obtained. The collected ants can be killed using a screw cup jar with a sponge containing cyanide or ethyl-acetate. This allows – mainly important for ants living in loose soil or sand – a more easy separation from soil particles after sampling with an aspirator. As another possibility the collected ants can be put directly into high concentrations of ethanol (70–99 %). Fast killing and a proper conservation is done with ethanol in a single process. Compared to 70–75 % ethanol which is frequently recommended as storage medium for insects, the higher concentration shows considerable advantages for mounting. Preparation from 70 % causes difficulties in appropriate adjustment and positioning of body parts. After they are brought in a desired position, they try to return into their original position when released. This feature often lasts for many hours – depending on the ant size – thus requires fixation until the glue has dried and leads to a high time expenditure (Seifert 2018).

2.2 Long-term storage

In general, ants are either stored in ethanol, ethanol-glycerol mixtures, or as dried mounted specimens in insect boxes. As the preparation of the collected ant material needs a high amount of time, sampling and preparation may be separated by a longer period of time. Thus, the storage and conservation liquid used are highly relevant for the results of later scientific work. Ethanol conserves via dehydrating the material, which usually, due to the strongly sclerotized exoskeleton, has only a small impact on the body shape of ants. The wet material has to be stored continuously in pure undenatured ethanol (99 %+) to conserve the DNA for several years (Post et al. 1993) as well as outer body parts for decades. Due to dehydration and the resulting water intake, solvents should be changed regularly to inhibit denaturation of DNA. Best conservation of outer body is given by using 10 % glycerol in ethanol. Glycerol does not evaporate, is hygroscopic and thus prevents desiccation of the material even if it is stored for long time periods. On the other hand, the main disadvantage of this method is the formation of a glycerol layer on the cuticle causing a sticking-together of pubescence hairs or masking of important micro-structures of cuticle and pilosity. This layer has to be removed by one or two washing steps. One unwelcome effect of ethanol is the strong bleaching effect leading to a loss of pigmentation after several years or, on rare occasions, it also leads to the opposite effect in converging reddish ants into blackish ants. Thus, the samples should be stored preferably in a cool and dark place in order to prevent additional bleaching by (sun-) light and evaporation of solvent. Formaldehyde or its ethanol dilutions also show high bleaching effects and an irreversible hardening of joints (Stephenson & Riley 1995). Additionally, due to the widely discussed issue of a potential risk for health, the use of formaldehyde should be avoided as far as possible (Hatchfield & Carpenter 1986). Plant material, soil particles, and other remnants of sampling should be removed. The latter may have an impact on the acidity of the storage liquid or may cause discolorations (Smithers 1981, Seifert 2007).

Ethanol collections are classically stored using glass tubes, which are closed by some cotton wool and placed in a second container filled with ethanol. This method has the benefit of an easy and fast refill, but leads to high searching effort if a single tube is of interest. Another option is the storage in plastic tubes with some sealing ring containing lids. These tubes do not necessarily need a second container and can thus be more easily stored in a numerical order. An additional digital file providing information on each number is an important step for well-arranged collections. Many of the sealing rings, however, do not fully ensure a tight closing, which may lead to a complete desiccation of the material after a longer period of time. The possibly best way of storage would be tightly closing borosilicate glasses, which inhibit chemical processes and interactions harming the conserved material – mainly triggered by sodium-ions from normal glassware or by oxygen from not tight closing lids. The development

of tight closing and cheap borosilicate glass tubes allowing storage without any outer container would be a revolution in collections. Theoretically, it would be possible to produce such glass tubes, but the high production costs presently seem to prevent the marketing of such systems.

Mounted specimens can be stored for an infinite time, but may still be destroyed by external factors such as moisture or pests. Thus, the collection room should have a low air moisture and low to moderate temperature to inhibit pest species as well as possible. For the control of pest organisms, pesticides – for example 'Vandal Mottenschutz' – are commonly in use, but should be avoided as far as possible due to potential health hazard. The material is best stored in tightly closed insect boxes of acid and formaldehyde free wood to properly protect the material (Hatchfield & Carpenter 1986). The insect pins should be corrosion-free and all labels and cardboards made of acid-free paper. Depending on the body-size, up to six specimens or morphospecies of one nest sample can be mounted together on the same pin – this may include males, gynes, or different worker castes that were found in the same (nest-) sample (Agosti et al. 2000). One specimen on one pin would lead to a much faster exhaustion of available collection space.

2.2.1 Appropriate labeling

Correct labeling is essential in a scientific collection. Each sample tube or needle with mounted specimens has to carry at least two labels: The first label with information on sampling date, locality, and collector as well as a second label which includes the species name, the name of the determinator and, ideally, the year of determination. Without information on the exact locality and sampling date the prepared material can be considered scientifically worthless. The second label is in case of type material of taxonomically high importance. The labels should be made out of acid-free, fine card stock or thick paper. A non-neutral pH of the material would cause deterioration within 40–50 years, resulting in brittleness or, in the worst case, detachment from the pin. Especially in geographic regions with a high level of air humidity the latter process is accelerated dramatically. India ink that is preferably used for writing labels has been a time-proven standard for centuries. The use of laser printers is basically an excellent modern option but the long-term stability of the printed information has to be considered for which there is little experience. A printed label – using a font without serifs – seems to be most suitable to ensure

Figure 1. Largest species *Dinoponera australis* Borgmeier, 1937 with smallest species *Discothyrea sexarticulata* Borgmeier, 1954 on its head. Photo: Guilherme Ide.

readability. Printed labels should have, for size relations commonly observed in ants, a font size of four to five points and a general cardboard size of 7–10 mm width and 15–20 mm length. This label size is compromising between a high amount of information and efficiency of space in insect boxes. An even number of written lines on the labels is recommendable not to obliterate important data by the pin itself. A right-alignment of letters could be a possible solution. Nevertheless, the labels should be consistently oriented – readable from the same direction in dependence of left or right handedness – parallel to the mounted specimens and should ideally not exceed two or three in number (Agosti et al. 2000). In addition, the labels may also serve as spacers towards other specimens or insect box walls in order to protect the pinned specimens.

3. Preparation of material

The preparation is often a compromise between stability and the best visualization of a maximum of characters. Especially for morphometrics, the lateral view on both sides and also on slightly hidden parts – for example the gula, the ventral or the inner side of the tibia – should be possible. For preparation of workers and gynes, the main focus is set on non-genital characters, whereas the copulatory organs are accessorily most important in males (Clausen 1938, Schlick-Steiner et al. 2006, Wagner et al. 2017). Furthermore, wing venation can be used in gynes and males for determination purposes but is of less interest from an alpha-taxonomic point of view (Klingenberg & Dietz 2004).

3.1 Preparation techniques for workers and gynes

Workers and gynes (Fig. 2, 3) can be prepared by using basically the same techniques. The main arguments for or against the use of a particular preparation technique refer to the respective size of the ant, the location of taxonomically important characters and the amount of individual previous practice. In case of alate gynes, wing preparation is best done by cutting the front- and hind-wings at the base and gluing them flat on the same or another cardboard to allow the best evaluation of wing parameters (Heinze et al. 2002, Schwarz 2014). For the workers (major, etc.) the following preparation techniques are frequently in use.

3.1.1 Pinning

The first classical mode of preparation was to put a thin insect needle directly through the middle of the mesosoma. This method was and is mainly done with very large species, the main advantage being the low time needed for preparation. However, the high fragility of the dried ant material in connection with the bending property of the needle all too easily leads to destruction (Koch 1956, Hölldobler & Wilson 1990).

3.1.2 Several ants on one plate

Another frequently seen method is to put some water-soluble glue on a cardboard and – depending on the respective size of the specimens – to fix one to eight ants on one cardboard. This method saves material, time and space, but the free view on many characters of the ant is blocked or these are masked by the glue. Accordingly, this mode of preparation is not recommended. Nevertheless, it is possible, even after a long period of time, to re-prepare the dried ants in another position.

3.1.3 Standing position

In general, the standing positioning (Fig. 2, 3) is highly recommendable for nearly all kinds of ants. The specimen is glued on its tarsae in a standing position on a paper plate. The fore-legs are orientated frontwards whereas the middle and hind-legs are directed backwards – aiming for a natural position of the legs. The mesosoma is uplifted to give an unrestricted view on the mesosoma as well as on the waist segments. The scapes are bent to the front and the antennae are glued parallel to the front-tarsae on the paper plate (Fig. 3). This technique ensures the highest stability with additional protection of the antennae. The latter benefit from their fixed position for measurement purposes. Additionally, characteristics of the legs are easily accessible – this is especially important, in the Central European fauna, for the genera *Formica* and *Lasius* (Seifert 1992, 2000). One disadvantage of this preparation mode is the rather large preparation time needed. In very large ants, where the relation between material stability of dried appendages and body mass becomes low, this mode of preparation bears the risk that the whole specimen may break off from the cardboard caused by percussions during mail transport as it is with the Wilson preparation. Furthermore, when very large ants are prepared from ethanol, the strong mechanical resilience of the stiff body parts may cause difficulties in adjustment of body parts – but this disadvantage applies to any mode of mounting (Seifert 2018).

Figure 2. Gyne of *Myrmica hirsuta* Elmes, 1978 in standing position. Photo: Roland Schultz.

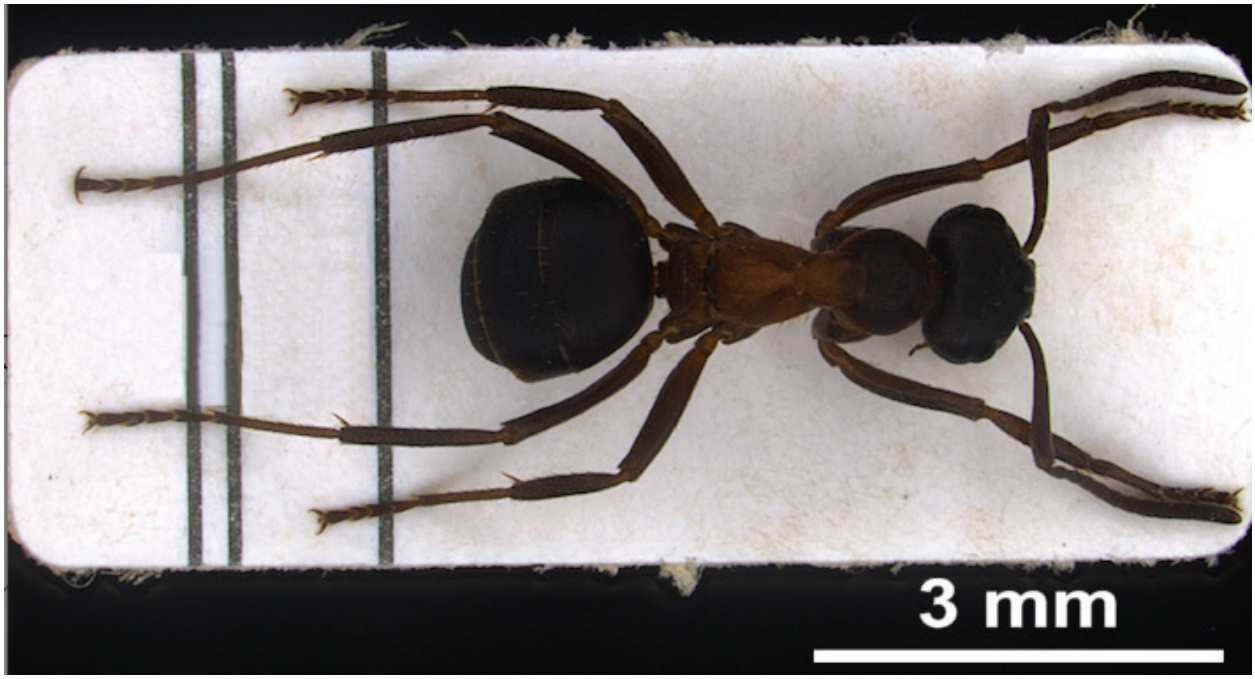

Figure 3. Top view on *Formica uralensis* Ruzsky, 1895 in standing position.

3.1.4 Preparation according to Wilson

The preparation method most commonly used is the so-called Wilson-preparation (Fig. 4). In this preparation type, the legs are bent in a ventral position using a fine forceps or a curved needle. The ant is glued between its second and third coxae on the tip of a triangular cardboard – according to the usual standard with the specimen‘s head pointing to the left when viewing the cardboard lengthwise from the pin side. After allowing the glue to dry, the legs and antennae should be adjusted in a position appropriate for measurement. The size of the triangular paper plate can be chosen variably with regard to the specific size and weight of the prepared specimen (Graser 1959, Hölldobler & Wilson 1990, Agosti et al. 2000). For improved stability it is recommended to use a thicker cardboard with a broader tip for large species such as *Camponotus* spp. This type of preparation has the advantage of a free view from nearly all angles, especially for characters belonging to ventrally situated mouth parts. One disadvantage is the obstructed view when looking from the cardboard/pin side. Thus, this preparation mode is not appreciated by taxonomists who apply morphometric determination and need a bilateral recording of characters. Furthermore, these investigators do not like the crosswise and more eccentric position of the specimen relative to the pin‘s center of rotation, which makes the handling and adjustment of the specimen more difficult during measuring processes with x-y-z stages. Another disadvantage is the higher risk of damaging the specimen already in weak collisions and of breaking off especially specimens with a larger body mass from the cardboard (Seifert 2018).

3.1.5 Mounting with basket-shape arrangement of legs

A technique currently used by some taxonomists who apply morphometric determination is a mounting method where the legs of the ant are bent basket-like on its ventral side and then glued with its metatarsae and tarsae on the tip of a triangular cardboard – parallel to its longitudinal axis (Seifert 2018). The antennae are bent downwards until they form a right angle to the middle axis of the head capsule. The big advantage of this method is the elevated position of head, mesosoma, and gaster relative to the cardboard and that the legs do not obscure the investigation of most characters placed on these body parts. Sometimes, a combination with other modes of preparation – for example according to Wilson – improves the view on ventral characters, which can have benefit for photography purposes (Fig. 5). The higher position of the ant as well as the free view from nearly all angles may highlight important characters. The only disadvantage compared to the other methods is the limited view on the legs. Accordingly, basket-shape preparation is clearly preferable only for those ant groups, in which, according to current knowledge, investigation of metatarsal and tibial characters has low taxonomic importance (Seifert 2018).

3.1.6 Preparation of *Chthonolasius* workers

Special, taxon-specific, preparation techniques may be necessary for proper character investigation of some ant taxa. For instance, in workers and gynes of the subgenus *Chthonolasius* an unrestricted antero-caudal view on the petiole is desired for taxonomical measurement and determination. Here, the junction between petiole and mesosoma is cut and the severed body part is glued by the gaster tip on the same cardboard in some distance from the mesosoma in a position allowing a free view on the full area of petiole (Fig. 6). The front body is prepared in standing position near to the tip of the cardboard. Compared to the other methods, this preparation is more time-consuming and it needs some experience and practice to prevent the surfaces from being stained by leaking body fluids when the specimens are prepared from liquid storage.

3.2 Preparation of males

Preparation of males often requires special treatment of the genitalia which are placed at the end of the abdomen. There are three different approaches of preparation. Firstly, males can be prepared after Wilson, which allows a proper view on the abdomen from all angles. In the second method, only the genital is cut off and is glued with its parts remaining in situ on the margin of the same paper plate. The rest of the specimen is prepared in standing position. This method allows a view on the genital from dorsal, ventral and caudal but needs some caution to prevent destruction or deformation of the genital structures. In a third method, the gaster can be cut off entirely – similar to the *Chthonolasius* workers – and is afterwards fixed in upright position (Fig. 7). This method is easier to perform, but it often leads to a less optimal result because the bending of the gaster may disturb the ventral view on the genital. All three methods provide a sufficient view on all parts of the body and on the genital in an in-situ position. Thorough investigation of shape and structure of the different elements or parts of the genitalia requires dissection after

Figure 4. Mounted specimen of *Camponotus vagus* (Scopoli, 1763) in a preparation recommended by Wilson.

Figure 5. A combination of a basket-shape arrangement of legs with a preparation according to Wilson applied on *Formicoxenus nitidulus* (Nylander, 1846).

maceration and preparation of slides for transmitted-light microscopy (Forbes & Do-Van-Quy 1965, Gotwald & Burdette 1981). The value of male genitalia for species separation is, according to present knowledge, significant in rather few ant groups (e.g., *Tetramorium caespitum* complex, Wagner et al. 2017, Schlick-Steiner et al. 2006). In several genera such as *Myrmica*, *Lasius* or *Formica*, species are much better separable by somatic characters

Figure 6. Special preparation of *Lasius nitidigaster* Seifert, 1996.

Figure 7. Prepared male of *Lasius niger* (Linnaeus, 1758) in standing position and cut gaster in upright position.

of workers than by genital characters of males (Seifert 1988a, 1988b, 1992, 2000; Seifert. & Galkowski 2016; Agosti 1989). Furthermore, males are available only for a short period of the year and show higher variation due to their haploid genome (Kulmuni et al. 2010). All these difficulties can explain that existing systems for male determination down to the species level are frequently unreliable and that keys for males are rarely provided (Wagner et al. 2017). Nevertheless, characters of males may be of significant use in the delimitation of groups of closely related species within genera such as in *Myrmica* (Radchenko & Elmes 2010) or *Tapinoma* (Seifert et al. 2017).

3.3 Preparation and dissection of inner organs and musculature

In addition to preservation of exocuticulary characteristics, sectioning and conservation of organs and musculature provides scientific important information – age, dispersal abilities, reproductive capability, etc. There are several commonly used techniques for the different body parts. Usually, a dish with dark colored paraffin wax is filled with 70 % ethanol up to a height of approximately 5 mm. Insect needles of appropriate thickness can be used to fixate material if necessary. The main disadvantage of sectioning is the destruction of material, which is then no more available for later mounting and taxonomic processing.

3.3.1 Preparation of wing musculature

Hymenopterans, in general, have indirect wing musculature which is characterized by dorso-ventral muscle strands for each wing. Another thick strand of muscles is located in the middle of the thorax in parallel orientation to the body axis. After the nuptial flight, the wings are dropped and the musculature is increasingly replaced by fat tissue (Jones et al. 1978, Keller & Passera 1989, Heinze et al. 2002). Thus, the measurement of body fat is a proper technique for age determination of gynes (Wagner & Gordon 1999). In order to give access to the musculature, the thorax of the fresh gyne is laterally cut underneath the wing insertions and subsequently spread using fine forceps (Fig. 8). The amount of musculature gives information about the dispersal tactic – for example rather small musculature in combination with small wings (brachypterous gynes) may hint to short distance dispersal and vice versa (Heinze et al. 2002).

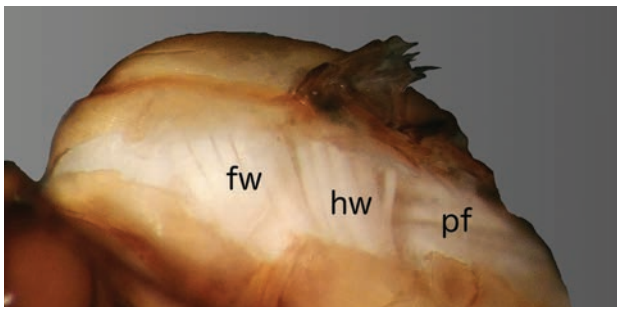

Figure 8. Sectioned young gyne of *Formica sanguinea* Latreille, 1798 with lateral view on the parallel flight musculature (pf) as well as the musculature of fore- (fw) and hind-wing (hw).

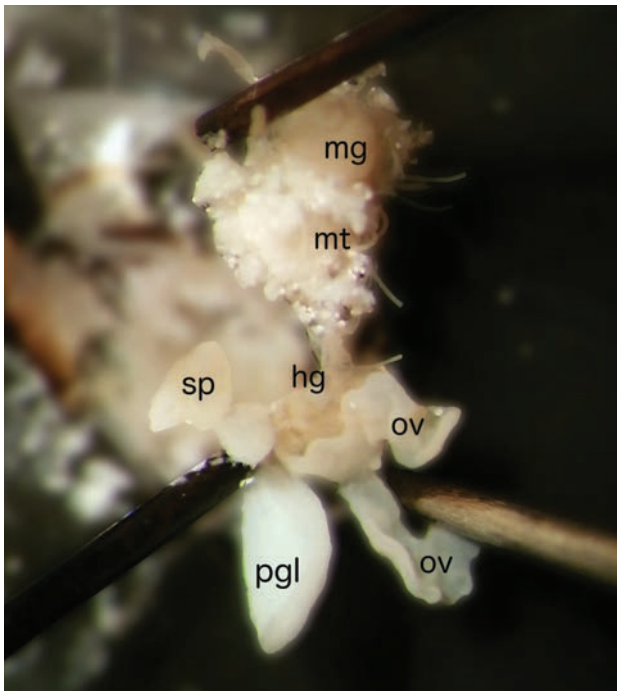

Figure 9. Section of *Formica sanguinea* gyne Latreille, 1798 giving view on mid-gut (mg), Malpighian vessels (mt), hind-gut (hg), spermatheca (sp), ovarioles (ov) and the poison gland (pgl).

Figure 10. Microscopic view on the spermatheca of *Polyrhachis dives* (Smith, 1857). Photo: Alfred Buschinger.

3.3.2 Preparation of the reproductive organs

The preparation of the reproductive organs (Fig. 9) – mainly with a focus on the spermatheca – provides valuable information about the reproductive capabilities of gynes or sometimes workers (Holliday 1903, Peeters & Higashi 1989). Furthermore, the relation between the number of ovarioles and the amount of sperm in the spermatheca allows estimations of the respective egg production capability (Tschinkel 1987). In the first step of preparation the end of the gaster is cut and the sclerotized abdominal rings are removed. Afterwards, the digestive tract can be spread and fixed with insect needles. The ovarioles and spermatheca, which are wrapped by other organs are separated using some fine needles and can be cut off for further investigations (Wheeler & Krutzsch 1992, Billen & Buschinger 2000). The spermatheca can, afterwards, be put on a slide for microscopic investigation (Fig. 10). The section of the reproductive organs provides good information but needs a lot of previous practice. Furthermore, it is of benefit to use fresh material for preparation since ethanol storage dehydrates the tissue, alters the typical shape, and leads to higher fragility of the tissue.

3.3.3 Preparation of (poison) glands

It is of high scientific value to understand the communication of the eusocial insects, for example in the two very important taxa ants and bees (Hölldobler & Wilson 1990). Thus, the compounds which are produced in the numerous glands (Fig. 11) have to be characterized and studied (Hölldobler & Engel 1978, Hölldobler & Engel-

Figure 11. Poison/Dufour gland of *Solenopsis* Westwood, 1840. Photo: Justin Schmidt.

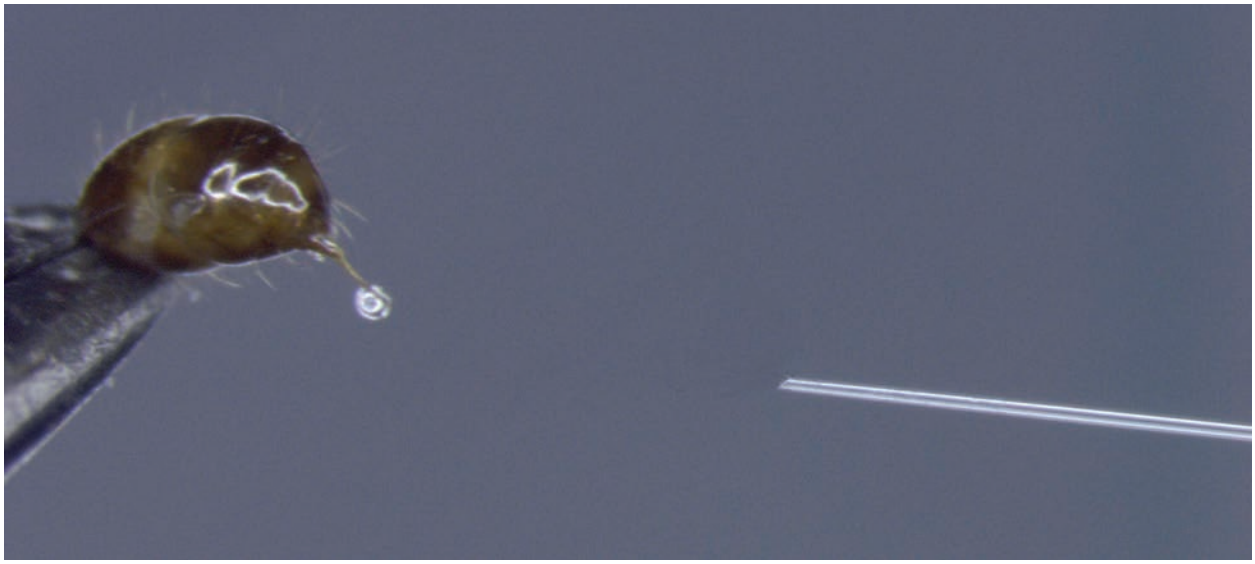

Figure 12. Milking of a fire ant *Solenopsis* Westwood, 1840. Photo: Eduardo Fox.

Siegel 1984). Furthermore, the hormones as well as the poisons and repellents – for example citronellal – can be of potential interest for pharmaceutical and biotechnological purposes (Poulsen et al. 2002). Sectioning and following extraction of special glands as well as milking (Fig. 12) is a common technique for understanding the produced chemicals. For the extraction of the poison and Dufour's gland, the gaster is carefully cut at the anus and all sclerotized parts are removed. The inner organs are spread and the glands are separated. For a proper investigation of the produced compounds it is essential to use fresh material which has not been killed and stored in ethanol. The ethanol would strongly wash out and dilute the compounds. Furthermore, the dissection is easier to be performed in fresh material – for similar reasons as described in the preparation of reproductive organs. An appropriate killing method for this purpose is the use of cyanide jars.

4. Conclusions

This article presented a comparing, descriptive, and illustrated summary of the most common techniques of ant preparation. It can be seen as a guideline for amateurs as well as for professionals with the goal of conserving natural history of ants in a proper way. In a scientific collection all kinds of preparations can be found due to the different preferences of the respective scientist. The individual previous knowledge on genus specific characters is also of high importance. Only myrmecologists who dealt with, for example *Chthonolasius* workers or persons who gained information from taxonomic papers would know the importance of the specific preparation and cutting technique. This lack of information may lead to inappropriate preparations which need to be re-prepared to have the full scientific use. The first methods of conservations, for example pinning of ant material, led to destruction or strong change of morphology. Such material – often being of outstanding value – is hard to re-prepare without risking further damage and lacks valuable morphologic information.

Within the last years, collections received attention due to their new developed function of being genetic archives (Berman et al. 2014, Lohaus & Van de Peer 2016). The next generation sequencing accesses whole genomes of collection material – thus also ant species. This allows studies on old ant material with taxonomic or population genetic purpose and might make collections and prepared ant material more valuable than ever. The access to type material can be, depending on its location, difficult or impossible. A sequencing of all type material with subsequent free use databases would represent an ideal goal of DNA taxonomy but will remain a very incomplete story as extraction of DNA from primary types of small-bodied, weakly sclerotized insects is often not possible without causing deformation of cuticle and concealing of very delicate surface structures.

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