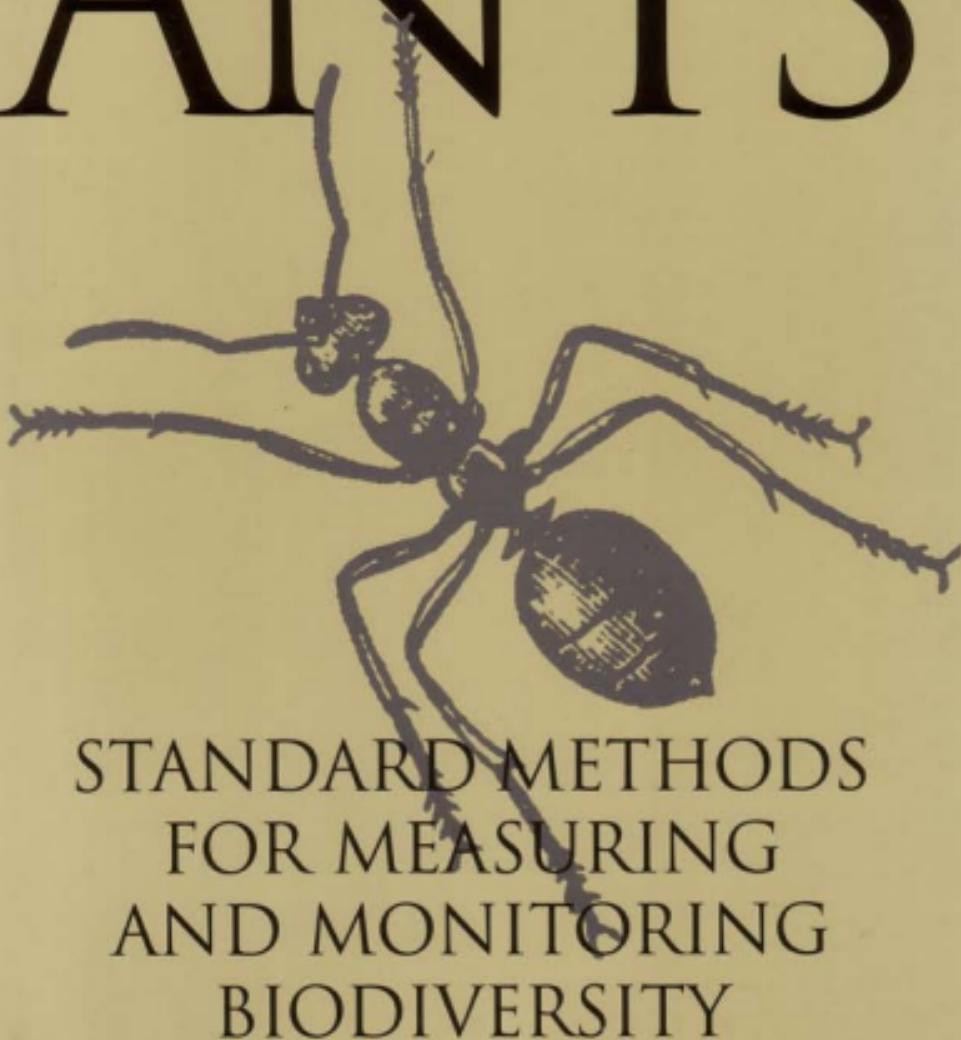


# ANTS



STANDARD METHODS  
FOR MEASURING  
AND MONITORING  
BIODIVERSITY

EDITED BY DONAT AGOSTI, JONATHAN D. MAJER,  
LEEEANNE E. ALONSO, AND TED R. SCHULTZ

FOREWORD BY EDWARD O. WILSON



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STANDARD METHODS  
FOR MEASURING  
AND MONITORING  
BIODIVERSITY



## **Biological Diversity Handbook Series**

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**Series Editor: Don E. Wilson**

This series of manuals details standard field methods for qualitative and quantitative sampling of biological diversity. Volumes focus on different groups of organisms, both plants and animals. The goal of the series is to identify or, where necessary, develop these methods and promote their adoption worldwide, so that biodiversity information will be comparable across study sites, geographic areas, and organisms, and at the same site, through time.

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This book is dedicated to the memory of William L. Brown Jr.,  
with affection, respect, and gratitude. For the inspiration you provided,  
for the firm foundation you built for ant systematics, and especially  
for your generous soul and irreverent good humor, we will never forget you, Bill.



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# Specimen Processing

## Building and Curating an Ant Collection

John E. Lattke



The proper preparation of ant collections is just as important as the collection of specimens in the field. Unlike some groups of organisms, such as birds and mammals, which can be identified in the field, ants must be carefully preserved and prepared for identification in the laboratory. The use of good preservation and preparation techniques is critical to collection quality and serves to facilitate the identification of species. Poor collecting and curation practices can substantially diminish the value of research collections.

This chapter outlines the process of assembling an ant collection and maximizing its value. The emphasis is practical, and the chapter focuses on a few issues critical to the maintenance of high collection quality. This discussion is intended to apply to large and small collections

alike. General aspects of keeping an insect collection are discussed in a number of texts (e.g., Martin 1977; Borror et al. 1989; Upton 1991), and the reader should consult these as well. We hope our contribution will provide food for thought for practicing ant systematists and ecologists alike. For newcomers to myrmecology, we hope to provide some help in ensuring the value of their contributions to museum collections, and to the study of ant systematics and diversity in general.

### Sorting Ant Specimens

The first step in ant specimen preparation is to sort the material collected from the field. After field work, ant specimens are usually contained in bags, vials, and jars and are mixed with soil,

bait, other organisms, and miscellaneous organic matter. Separating ant specimens from such a mix can be tedious work, but methods do exist to permit a faster search by eliminating the dirt and mineral matter from the sample. It is important to remove the ant specimens from this other material as soon as possible in order to prevent damage to specimens by abrasive particles and to avoid the formation of coatings of clay and mineral salts on specimens.

During the sorting process it is extremely important that the collection data, particularly the field number, always be kept with the specimens, and that the samples not be pooled before all have been identified. It is best to prepare and place labels in the vials and petri dishes first when working with samples.

### Salt Water Extraction

The salt water method of extracting ant specimens from other materials is highly recommended for its simplicity and low cost. The process is simple. Slowly heat water in a beaker on a hot plate, adding generous amounts of salt until the solution becomes saturated and no more salt will dissolve. The water should be hot but not scalding, and never boiling. Empty the sample with ants (e.g., a vial) into a graduated cylinder of no more than 4 cm diameter and drain off the alcohol. Add the saline solution to the sample, cover the top, and slowly turn the cylinder upside down a few times. Dirt and other inorganic material will sink while most organic material, including the ant specimens, will float to the top. Tapping the cylinder will help to dislodge larger items that may be suspended by air bubbles or have adhered to the sides of the cylinder.

Allow 15 seconds for settling, then quickly decant floating matter onto a fine mesh of plastic or a metal strainer and rinse it with alcohol. Repeat the process two or three times, rinsing well with alcohol each time. Take the material from the mesh and place it in a petri dish with

alcohol. Then sort the ant specimens from the other organic material. Sometimes plant parts, such as roots or bits of decayed wood, will still abound, but at least the dirt and sand will be gone.

The material remaining at the bottom of the cylinder should also be checked by rinsing it well in a strainer, as heavier ants and other insects sometimes may sink to the bottom. If alcohol is at a premium, the initial rinsing of specimens may be done with warm water, but alcohol rinsing should be performed before storage of the separated specimens to avoid dilution of the alcohol in the storage vial and the danger of deterioration.

### Manual Sorting of Ants from Debris

Either after salt water extraction or directly after the collection of field samples, some manual sorting of ant specimens will be required. Manual sorting is usually performed with the aid of a dissecting or stereoscopic microscope. Initially the field samples (including ant specimens) should be poured into a petri dish and spread out, forming a layer that does not totally obscure the dish bottom. Alcohol can be added to the sample to dilute the mixture. After spreading out the sample, one may choose to wait about 15 minutes for the silt and sediments to settle if the solution is too cloudy. The dish is then inspected by systematically viewing each part under a microscope. A petri dish with a grid greatly facilitates the task; alternatively a piece of paper with a grid marked on it can be taped to the bottom of the dish.

Ant specimens should be manually picked out with forceps and transferred to individual vials of alcohol. Ants should preferably be handled with soft forceps or watchmaker's forceps, taking care not to squeeze them excessively in order to avoid damage. Sorting can also be done with a fine brush.

## Separating Ants from Other Arthropods

Since a number of insects and other arthropods mimic ants and may cause confusion during the sorting process, we recommend collecting and storing all arthropods that resemble ants for later verification. Several groups of wingless Hymenoptera (wasps and other groups) may be confused with ants. Identifying these specimens may pose a problem later on in the identification process, as keys to ant subfamilies and genera will not work. *Hymenoptera of the World: An Identification Guide to Families* (Goulet and Huber 1993) is an excellent help in determining whether a specimen is or is not an ant. Other insects or invertebrates found in the samples should also be removed from samples and stored separately for future study by other researchers.

## Identifying Ant Specimens to Morphospecies

Since biodiversity data are often analyzed by relying on the presence or absence of species, accurate sorting and identification of ants at the species level is important. Although there have been substantial advances in taxonomic work on ants over the past decade, identification of tropical ant specimens to species is still challenging owing to the lack of identification aids for many genera. Limited availability of relevant literature, lack of recent revisions and expertise in some groups, and the backlog of work for most ant specialists make the recognition of morphospecies by nonspecialists a necessary part of biodiversity studies (see, e.g., Beattie and Oliver 1994).

Identification of ant specimens begins with dividing specimens from each sample into morphospecies. Individuals are grouped into morphospecies according to distinct morphological characteristics without reference to taxonomic classifications.

The challenges involved with species and morphospecies identification vary with the

scope of each project. One advantage for many non-ant specialists working with environmental monitoring, conservation evaluation, and ecological research is that they will be dealing with little geographic variability in morphology because of the sampling of relatively small areas. Taxonomists generally must look at material covering the whole geographic ranges of species and genera, and must deal with greater variation. Although Oliver and Beattie (1996a) found little difference between ant morphospecies determined by nontaxonomists and biological species determined by a specialist, their sampling methods tend to underestimate this difference.

Sorting to morphospecies will involve the use of a stereoscopic microscope. A good-quality scope as well as good illumination will help make long hours of staring through the lens more bearable and decrease the chances of error due to eye fatigue.

Initially, ant specimens from each sample should be put into a single vial. Then, depending upon the number of ant specimens in the sample, the sorted material can be divided into separate vials for future work, or it may all be sorted to morphospecies in one session. Initial sorting is usually conducted in petri dishes. Putting material into petri dishes before putting it away into vials permits easier control by the principal researcher.

Although it is possible to identify ant species in alcohol-filled petri dishes, it will generally be best, and it is strongly recommended, to mount a series of three specimens of each of the species in question. Distinguishing characteristics of ant species can best be viewed on dry, mounted specimens, for they are often masked by the alcohol. At least three to ten ants from each morphospecies should be mounted to document a geographic record, depending on how many samples are being collected. If some common species are present and dominant, it is not necessary to mount more than three specimens

per sample or survey. It is strongly advised that all the remaining, nonmounted specimens be kept in one vial, in case taxonomic problems emerge later. It is not necessary to keep each sample in a separate vial; all the samples from one survey can be combined into one vial.

Specimen mounting and morphospecies sorting will go hand in hand, and it may be necessary to go through several cycles of separation, mounting, and comparing to make sure that all the material has been accurately sorted. Ideally at the end of the sorting one should have mounted specimens from each species present in the sample, even though the vast majority of specimens will remain in alcohol.

The time and resources invested in such a project will depend upon the scope of the project and its objectives (Chapters 10, 13, and 14). If the study involves the separation and counting of every ant and the mounting and labeling of representatives of each species, a principal researcher and a full-time assistant will need at least a month of work for 50 mini-Winkler samples with abundant and diverse ant material (B. Fisher, pers. comm.). If only species numbers are necessary, two trained part-time students can sort and mount 50 samples within a week (J. Delabie, pers. comm.).

## Mounting Ant Specimens

The proper preparation of mounted ant specimens is key to identification. Poorly mounted specimens can rarely be identified because diagnostic characters are frequently obscured by other body parts or by glue. The following protocol describes the preparation of a standard mount that facilitates examination of the specimen and enhances its value. The technique requires practice to accomplish successfully, but the resulting specimens, easy to compare and examine, make the time and effort worthwhile. Direct pinning of ants (putting the insect pin through the ant body) is not usually done,

except for the largest of ants, because of the propensity of the ant cuticle to fracture when penetrated by the pin. Mounted specimens can be stored indefinitely if kept away from moisture, temperature extremes, light, and insect pests.

For small ants, three specimens from the same nest series, or three specimens from the same morphospecies, may be mounted together on the same pin. These three specimens should preferably be of different castes (e.g., worker, soldier, queen). Space must be left on the pin below the specimens for the locality data labels. It is advisable to include a note on the label indicating the origin of the specimen (i.e., nest series or traps).

Learning this technique can be difficult, and it is recommended that trainees first practice with large specimens and then learn to mount progressively smaller ants.

Ant specimens are prepared by mounting them on a paper triangle, generally referred to as a point (Fig. 11.1). Ants are glued to the tip of a small triangle of stiff cardboard or bristol board of neutral pH. Dimensions of the triangle should be no more than 10 mm long and circa 2 mm wide at the base. Points can be easily made using a specialized point puncher (similar to a paper hole puncher) available from entomological supply sources. A water-soluble glue should be used; it should be stored in a sealable petri dish while working to avoid excessive drying.

The selected specimens are taken out of the alcohol and manipulated with fine forceps so the legs are directed ventrally and away from the body. With the specimen's head facing toward the left the ventral areas of the meso- and metacoxa are left relatively exposed so the tip of the triangle can easily touch them. Before gluing they should be put on absorbent paper to dry. Then the series of ants, duly associated with their collection data, may be organized into columns or rows on an index card for expedient, assembly-line processing.

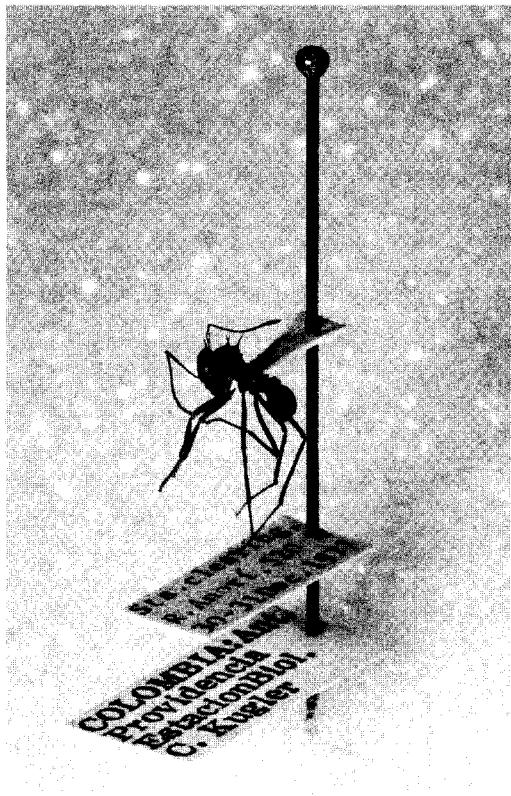


Figure 11.1. An ant mounted on a point and pinned with labels. Photo by Ted Schultz.

A tiny amount of glue is placed on another index card, the triangle is grasped by the base with forceps, and a small amount of glue is allowed to adhere to a single side of the apex of the triangle. The smaller the ant, the smaller the amount of glue needed. Alternatively, one may use two types of glue, one thinned with water for use with small specimens and the second full strength for larger specimens. The tip of the triangle is then delicately maneuvered through the upward-pointing legs so as to touch only the meso- and metacoxa and glue the specimen. It will be necessary to mount small ants using a stereoscope. Once the glue is dry the triangle with the ant is picked up with forceps, and an entomological pin is run through its base, taking

care not to bend the triangle or damage the specimen.

An alternative method is to mount ants directly onto a prepinned triangle. Using this method, up to ten ants can be spread with their heads to the right (as seen from the researcher's point of view), and if possible with the legs spread out. A cardboard triangle is first positioned on a pin (Fig. 11.1). Then the pin is stuck into a piece of cork or Styrofoam and placed under the microscope so that the tip of the triangle is clearly in view. A fine drop of glue is then put on the tip of the triangle, just big enough to glue the ant's alitrunk (thorax) on the tip. The ant is picked up with two pairs of forceps, one holding the right front leg and the other holding either the left or right hind leg. The ant is then set on the triangle, so that it is at the very edge of the tip and the petiole is freely visible. Depending on the consistency of the glue, the specimen may be held for few seconds to prevent it from falling off the pin. The legs should be arranged so that the researcher has a full view of the alitrunk outline and the ventral part of the petiole, but care must be taken not to pull off the legs in the process.

The finished product is an ant transversely mounted on the apex of the triangle, with its head pointing to the right when the triangle is pointing away from the researcher. Its head, waist, and gaster project freely and the ventral surfaces are visible; its legs should be directed downward so as not to obscure the rest of the body (Fig. 11.1). If the glue is dry it may be possible to manipulate some parts of the ant's body into a flat plane, although if the specimen itself is totally dry this may break it. Broken parts are preferably glued onto the same triangle or onto a separate triangle on the same pin. Trying to reglue parts together will usually result in the specimen being obscured by a mass of glue.

Dirt on specimens may hinder observation. It can be removed by briefly dipping the specimens into dilute acetic acid or potassium

hydroxide solutions. This will remove most mineral salts and oily residues, but ants must be thoroughly rinsed in water immediately after exposure to cleaning solutions. Organic solvents (e.g., xylene, toluene, hexane) remove oily residues but are dangerous to inhale or contact. These compounds should be used only in well-ventilated areas or under a laboratory hood while wearing gloves. Strong soap solutions can be useful for oily residues, but once again thorough rinsing afterward is necessary.

## Dissecting Ant Specimens

Dissections of ants are sometimes helpful in order to obtain a good understanding of the body parts. When making a dissection, first soak the ant specimen in 10% potassium hydroxide solution at 80°C (for one to several minutes, depending on size and degree of sclerotization, until the ant falls to the bottom of the vial), then rinse several times with water and transfer the ant to 70% ethanol, which prevents it from floating during dissection. After dissection, put the dissected parts into 100% ethanol and, if the parts are very delicate, rinse the ant with xylene, which hardens the cuticle. Finally, dry the parts on a piece of paper towel and mount the parts on a cardboard triangle so that the important structures point toward the viewer. For minute preparations, special tools may be constructed using minutiae (very fine entomological pins used for small insects) mounted on toothpicks or wooden matches; the tips of the minutiae can be slightly bent to form hooks or other useful shapes.

For labial and maxillary palp counts, it is sometimes necessary to dissect the mouth parts out of the head. This is best done with the specimen in ethanol. The mouth parts should be dissected out from the ventral side of the head, with one pair of forceps holding the head and the other gliding with the two arms of the forceps along the side of the buccal cavity (mouth

opening) into the head capsule, then holding firm to the mouth part, which will not easily be damaged by pressure as it is torn out. The wet mouth part is then put on a piece of paper towel and allowed to sit until all the ethanol is evaporated. During that time, it is best to manipulate the mouth parts so that all the palps are sticking into the air. This is also the best time to count the segments. The mouth part is then mounted on the same cardboard triangle as the head. The palpal count can then be noted on a colored (preferably green) label in the form "PF = 5,4" (palpal formula = 5 maxillary and 4 labial palps).

## Labeling Ant Specimens

Labeling is perhaps the most important part of specimen preparation. Without the pertinent field data presented on labels, biological specimens are worthless. Its label is basically an abstract of the most vital locality data for each ant sample.

## Materials

Labels should be written on fine, 100% rag or neutral-pH card stock, similar in gauge to that used for index cards, that will not let ink bleed. Many apparently fine card stocks may be cheaply purchased at stationary and office supply stores, but those not of a neutral pH will start to deteriorate on point-mounted specimens within 40–50 years and become brittle, easily breaking and falling apart upon manipulation of the specimens. Avoidance of such unsuitable stocks is especially critical for collections in humid tropical areas.

India ink is the time-proven standard for writing labels, but excellent labels can also be printed with a laser or dot matrix printer capable of making letters 4 or 5 points in size. Laser-printed labels are apparently safe for point-mounted specimens but should not be used for specimens preserved in alcohol, although dot matrix print-

er labels are adequate for this purpose. (Nevertheless, each new brand of print cartridge should be tested beforehand.) If labeling is to be done manually, the handwriting must be legible! Accurate locality data can be rendered useless if the information cannot be read.

### Label Size

For point-mounted specimens the labels should be approximately 7 mm wide and 15 mm long. The size reflects a compromise between the amount of information to be included and the ability to store and manipulate the specimens, as excessively large labels can easily damage other specimens.

### Position and Order of Labels

Ideally a pinned specimen should have no more than two or three labels. For mounted ants, labels are pinned underneath the ant specimens (Fig. 11.1). Labels must all be consistently oriented parallel to the longitudinal axis of the point or pinned specimen in such a way that one does not have to change the direction of the storage drawer with each specimen label to be read. The standard for point-mounted ants is that the label is read with the point directed to the left; for specimens directly on a pin the label is read with the specimen's head directed to the left. When perforating the label with the pin, it should be jabbed close to the right-hand margin for point-mounted specimens and close to the middle for directly pinned specimens. In both cases care should be taken not to obliterate important data with the pin itself.

For specimens preserved in alcohol, labels can be inserted directly into the vials with the ants. Multiple copies of labels in alcohol vials are helpful when additional specimens are to be removed for mounting.

### Label Information

The principal label (uppermost on the pin) should have no more than five lines and should

VZLA, Sucre  
Las Melenas 9.7 km NW  
800 m, 10° 41'N, 62° 37'W  
10-V-1993, J. Lattke, leg 4457

Primary Forest  
Leaf litter, Winkler

*Atta laevigata*  
det. 20-V1-1993, E. O. Wilson

Figure 11.2. Sample labels (not to scale) for mounted and alcohol specimens.

include the following standard information in this order (Fig. 11.2):

First line: country, state, department or province (abbreviated)

Second line: specific locality

Third line: altitude, latitude and longitude

Fourth line: date, collector, collection number

Brevity dictates that the country, state, and locality be abbreviated; standard abbreviations that can be easily found in gazetteers should be used. An abbreviation should not be so truncated that someone else will have difficulty in interpreting the name. The locality is the descriptive name of the collection site. A site can also be pinpointed by its direction and distance from a more prominent reference point, such as a large town. In abbreviating the date, the month should always be expressed as a Roman numeral and the year cited in full. The top label in Fig. 11.2 provides an example.

A second label, pinned underneath the principal locality label, may contain ecological information, such as habitat type and micro-habitat description (e.g., vegetation, rotting log), and the collection method (Fig. 11.2). Keep in mind that additional information can be accessed from the specimen database or notes through the collection number. Species and morphospecies identifications, the name of the person who identified the species, and the date identified should be presented on the last (second or third) label, since identifications may change but locality and collection data do not (Fig. 11.2).

### **Associated Data Records**

The best and safest storage of information on specimens is in publications based on the specimens and in which they are referred to using unique field data or sample numbers. All collection data should also be entered into a specimen database.

## **Identifying Ants to Subfamily and Genus**

Before ants can be separated into morphospecies, two important steps must be followed. First, knowledge of the important features of an ant's external anatomy must be confirmed before ants can be separated and identified. See Chapters 5 (especially Fig. 5.1) and 12 and also Hölldobler and Wilson (1990) and Bolton (1994) for basic descriptions of ant morphology.

Second, ant specimens, already sorted to morphospecies and mounted, are generally first identified to subfamily and then to genus before they are sorted into morphospecies within each genus (i.e., "morphospecies 1" will become "genus X morphospecies 1"). Identification up to the genus level and the most common morphospecies quickly becomes an easy task. Excellent keys to ant subfamilies and genera of all parts of the world are available and are fairly

easy to use. Resources for determining ant genera are listed in Chapter 12.

## **Separating Morphospecies Using Characters**

General recommendations are given in this section to assist nonspecialists in search of differences for species separation as well as for managing specimens and facilitating comparisons. They are all merely suggestions and should not be interpreted as a cookbook recipe, since the criteria for determining a species in one genus may not be valid in another. Criteria for species determination can differ from genus to genus, and the ant taxonomy literature should be consulted (Chapter 12) for clues to the criteria most commonly used, even if the references are not recent or from the same geographical region. Many morphological characteristics, especially in the case of older literature, may not necessarily be correct, but at least the results will be consistent with what is known, and they may eventually be corrected when revisions are carried out.

It is also strongly recommended that each worker keep his or her own notebook where characters used to separate each of the morphospecies are recorded. Ideally the notes should also include sketches of traits that are difficult to describe, such as hair patterns or shapes.

### **Morphological Differences between Castes**

Morphological differences between ant castes (workers, soldiers, queens, females and males), as well as polymorphism within castes, create an additional challenge for ant identification. Collecting entire nest series—containing all sizes of workers, soldiers (if present), females, and males—is the best way to learn about the variation within an ant species and to relate the castes to one another. Especially in local sur-

veys, this allows one to match all the different castes fairly quickly, and it also adds a measure of satisfaction to the collecting process, as an understanding of the biology of the species begins to develop. For many species, one can obtain samples of other castes, such as males and soldiers, by keeping a fraction of the workers and some brood from a nest in an artificial colony in the laboratory.

Specimens collected in pitfall traps or Winkler samples are often difficult to associate. In some cases it is relatively easy to match workers with soldiers and queens, but sometimes the castes look totally different. In general, the following guidelines apply:

*Queens* generally will resemble the workers, especially the majors, to an extent that pairing them from a non-nest sample is usually not problematic. Typically one finds that a queen has larger compound eyes, ocelli, a larger mesosoma with more segments and sutures as well as wings or wing stumps, and usually a larger gaster than the workers. Differences in sculpturing are usually not very great.

*Males* are wasplike and dissimilar from their female counterparts, so they are usually difficult to match with their conspecifics when taken disassociated from their nestmates. Normally males have much larger eyes, a short antennal scape, a small head relative to the mesosoma, and an elongate gaster (often with the genitalia protruding from the apex). It is often impossible to identify the males even to genus level. Currently there are no good keys to ant males.

In the case of *worker* polymorphism it is usually the major (larger) workers that furnish the most reliable characteristics for species separation, because the minor castes of some species from the same genus may present negligible differences among themselves. Workers from incipient (newly formed) nests on average are smaller than those from mature nests and have lighter coloration. Table 11.1 lists the genera in which polymorphism is present in the workers.

**Table 11.1** Ant Genera with at Least One Species in Which the Worker Caste Is Divided into Physical Subcastes<sup>a</sup>

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Ponerinae: <i>Megaponera</i>
Myrmeciinae: <i>Myrmecia</i>
Dorylinae: <i>Dorylus</i> , <i>Ectiton</i>
Ectoninae: <i>Cheliomyrmex</i> , <i>Labidus</i> , <i>Nomamyrmex</i>
Pseudomyrmecinae: <i>Tetraponera</i>
Myrmicinae: <i>Acanthomyrmex</i> , <i>Acromyrmex</i> , <i>Adlerzia</i> , <i>Anisopheidole</i> , <i>Atta</i> , <i>Cephalotes</i> , <i>Crematogaster</i> , <i>Dacetin</i> , <i>Machomyrma</i> , <i>Messor</i> , <i>Monomorium</i> , <i>Oligomyrmex</i> , <i>Orectognathus</i> , <i>Pheidole</i> , <i>Pheidologeton</i> , <i>Pogonomyrmex</i> , <i>Solenopsis</i> , <i>Strumigenys</i> , <i>Zacryptocerus</i>
Aneuretiniae: <i>Aneuretus</i>
Dolichoderinae: <i>Azteca</i> , <i>Iridomyrmex</i> , <i>Liometopum</i> , <i>Tapinoma</i>
Formicinae: <i>Camponotus</i> , <i>Cataglyphis</i> , <i>Euprenolepis</i> , <i>Formica</i> , <i>Gesomyrmex</i> , <i>Melophorus</i> , <i>Myrmecocystus</i> , <i>Myrmecorhynchus</i> , <i>Notostigma</i> , <i>Oecophylla</i> , <i>Proformica</i> , <i>Pseudophomomyrmex</i> , <i>Pseudolasius</i>

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<sup>a</sup>From Hölldobler and Wilson (1990:318).

## Choosing Characters

A quick overall look at an ant's body will usually permit preliminary separation of specimens using obvious traits, such as size, color, presence or absence of denticles, structure of the petiole and postpetiole, and odd mandibular shapes. This step permits the division of large samples of specimens into smaller, more manageable, lots. One can expect to find some variation in almost any trait, and trying to assess the limits of infraspecific variation is the crucial task for fine sorting. Color can be quite unreliable, so it should always be used in combination with other characteristics and not by itself. The final sorting calls for attention to finer anatomical details that will mean looking at more restricted areas of each specimen.

Rarely will one find a morphospecies that can be distinguished on the basis of just one outstanding character; a combination of three or more is usually needed. To help keep track of the morphospecies one may write down diag-

nostic characters or illustrate them on an index card. It is generally a good idea to keep a running list of the characteristics used to separate morphospecies so that specimens from different samples can be compared and grouped.

Study each body part from different angles in an effort to detect useful characters, such as shape, projection, excavation, pilosity, sculpturing, sutures, and sulci. To have a clearer image of any general body shape, it helps to use background lighting, so that only a silhouette is seen. Dirty specimens may present totally distorted silhouettes and sculpturing patterns—a common source of error. Cleaning may uproot pilosity, and care should be taken with the use of hairs as characters in such a case. Pilosity may differ between species, but more delicate hairs may be easily abraded, reducing their diagnostic value in some cases. When studying pilosity it helps to distinguish between the very short, fine hairs that form a base pilosity and the longer emergent hairs; their angle of inclination (using the cuticular surface as a reference) may be characteristic.

Some sculpturing may be difficult to assess because of a shining surface that reflects too much light. A strip of Mylar or chalk paper placed very close to the specimen between the light source and the ant will reduce glare and permit distinction of details of cuticular sculpturing. When working with lengths and widths, a comparison between the dimensions of one body part and another is helpful; this is why indexes are often used in keys and descriptions. A *Glossary of Surface Sculpturing* (Harris 1979) is a good introduction to the various types of sculptures and their terminology.

### Specific Characters

Figure 5.1 provides a general diagram of the body parts of an ant.

**HEAD.** The shape of the head itself may be distinct: Is it wider anterad than posterad? Are the

compound eyes part of its silhouette or not? Look for the presence of distinctive sculpturing that affects the cephalic shape, such as spines or crests. Mandibles are frequently useful for specimen determination. The amount and disposition of dentition should be noted, but dental abrasion does occur in older specimens. The clypeus frequently presents useful characters, and studying differences such as the shape of its anterior margin and sculpturing is recommended. How far back does it extend between the antennal lobes? Antennal scapes can differ in their relative length to the head; see how far back they extend beyond the posterior cephalic border. This distance can be quite obvious in some specimens, or one may gauge the distance in apical widths of the scape itself in close calls. The frontal carinae may have a distinct shape, especially the external margins; how far back do they prolong themselves? The eyes may differ in shape, size, and position on the head. Their presence or absence may vary within a species, such as in the army ants (ecitonines), where the minors are eyeless but the majors may retain a single-faceted eye. The ventral side of the head may reveal differences in the hypostomal teeth. The occipital corners frequently have distinct dentition, lobes, or other sculpturing of use.

**MESOSOMA OR ALITRUNK.** Each of the four parts (pro-, meso-, and metanotum and propodeum) that make up the mesosoma may have differences in sculpturing from the others even though impressed lines do not clearly separate them. In a dorsal view take note of the presence or absence as well as the development of the promesonotal suture and metanotal sulcus. The shape of the mesosoma in lateral view is a useful indicator of differences; note convexities and angles between different parts. The pronotum may present angles or denticles along its ventral margin that may not at first be noticed. The anteroventral edge of the mesopleura is frequently bordered by a carina that may present

differences in height and width. How are the propodeal spiracle openings oriented? What is their diameter or position on the body? A frequently overlooked spot is the declivitous propodeal face when it is surrounded by teeth. The legs may differ by the amount of pilosity or in the ratio of their length to width, and they may sometimes have characteristic spines on the tibia or tarsi.

**PETIOLE AND POSTPETIOLE.** The petiole will vary in the presence or absence of a peduncle and in its general shape. The length-width ratio of the petiolar node in a dorsal view may be helpful. The subpetiolar process—a lobe or denticle that may or may not be present on the anteroventral petiolar border, and that can be shaped in various ways—is frequently hidden from view by the legs.

**GASTER.** Differences in shape, especially in lateral view, may be quite distinctive, as may sculpturing. The ventral area close to the union with the postpetiole, as well as the rest of the first gastric sternum, may have useful characters that are difficult to observe owing to the closeness of the postpetiole (or more usually because of sloppy mounting). Attention should be paid to differences in sculpture between the basal and apical parts of the same tergite.

## Identifying Ants to Species

Identification of ant specimens to species, beyond morphospecies, is a science in itself, one that may take up to 10 years of study and practice to master. Fortunately, many studies of ant diversity can be carried out without species identification (Chapter 7). If one's study is limited to a geographically restricted area, identification to morphospecies, for which the genus name is secured, should suffice. For analyses over larger areas, species identification is important in order to make comparisons.

Without accurate species identifications, one would have literally to compare all the morphospecies from multiple research groups.

However, identifying ants to the species level will greatly enhance any ecological study by linking the species richness and diversity data to biological data on the species of interest. Species names are like gateways to the enormous amount of knowledge accumulated in publications over the years. In many cases, a species name allows one to associate the specimen and diversity data with information on the biology, ecology, or distribution of the species. It has recently been argued that without ecological and biological information about the species under study, interpretation of species diversity data is incomplete and may even be misleading (Lawton et al. 1998; Goldstein 1999). For example, several studies have found that ant species richness often increases with increasing levels of habitat disturbance but that the composition of the ant species changes (Chapter 7). Without knowledge of the biology of the species sampled, these changes in species composition cannot be interpreted.

A species name also allows one to search digital and printed databases, such as Formis (Porter 1999), with over 20,000 bibliographic records, or the social insects Web site (Chapter 1). This information enables further analysis and interpretation of the species data.

Before diving into species identification, one should be certain to have familiarized him- or herself with the basic morphology of ants (Chapter 5; Hölldobler and Wilson 1990; Bolton 1995b). Those who may still feel insecure should begin by choosing some of the larger ants from various groups and preparing drawings to compare various parts.

Species identifications are facilitated by first preparing a list of species known to occur in the area of interest. This can be done using the catalogue of the ants of the world (Bolton 1995b), which allows one to search for the type locali-

ties by country, through lists of local faunas, or by visiting local or regional collections and going through their records. This quickly becomes an enormous task, but the more data that are compiled and shared, the easier it will be for the next generation of inventories to proceed. Local lists can be found on (and should be submitted to) the social insects Web site ([http://research.amnh.org/entomology/social\\_insects/](http://research.amnh.org/entomology/social_insects/)).

## Assistance from Ant Taxonomists

Relationships developed with a major ant collection or with an ant taxonomist will undoubtedly make identification of ant specimens a much easier task. It is inevitable that a researcher will eventually accumulate specimens that should be looked at by an ant taxonomist in order to clarify dubious identifications. Before proceeding to send a large number of

ants to a specialist, one should first contact that person and ascertain if he or she has the time to examine them. Chapter 12 lists institutions with practicing ant taxonomists. Given that taxonomists are usually inundated with material, one will increase the chances of cooperation if the sample size is reasonable, the deadline for the determinations is not too tight, and duplicates are supplied, enabling the specialist to deposit them in his or her own institutional collection (Table 11.2). It is always a plus when the specimens happen to belong to the taxonomist's particular group(s) of interest.

It is best to first develop a synoptic collection of ants from the study before sending any specimens to a specialist for identification. The specimens should be sorted to morphospecies, and then only a few specimens of each morphospecies should be sent. If one has little prior understanding of ants, it might be wise first to send specimens of the ten most common

**Table 11.2** Rules for Submitting Specimens for Identification<sup>a</sup>

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1. Do not assume that specialists desire more specimens for their own sake; usually they are interested only in establishing new records or in additional species. The specialists are spending valuable time to make identifications. At consultant wages, this could be expensive. Therefore a request for identification is equivalent to asking for a substantial donation of time, either from the individuals involved or from their employing institution.
  2. Never ship specimens for identification without making prior, detailed arrangements with the specialist, including:
    - a. Return shipping costs.
    - b. Time frame within which specimens are to be returned.
    - c. Number and identity of specimens to be retained by the specialist.
    - d. Where type specimens will be deposited (if applicable).
  3. All specimens submitted for identification must be:
    - a. Properly prepared and preserved.
    - b. Provided with exact locality data.
    - c. Sorted to genus if possible.
  4. Never send bulk collections or unsorted collections with a request that the material be picked over to find things of interest.
  5. Remember that a refusal to make identifications of large masses of material does not necessarily indicate a lack of interest, but sometimes merely a lack of time or facilities.
  6. Under certain circumstances, a specialist may request a fee for providing an identification. This practice has become nearly universal for court cases, commercial activities such as pest control operations, or environmental impact studies. Make sure that both you and the specialist agree on a fee, if any, before the identifications are made.
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<sup>a</sup>From Arnett and Samuelson (1986).

morphospecies from the study. This approach will not overwhelm the specialist, and it will also allow time to adjust the method of preparation to meet the requirements of the specialist. This procedure will result in early identifications for the most common ants. It also shows that one is willing to spend some time sorting through the material and is willing to invest one's own scarce time in identification as well. Most taxonomists will not touch material lacking collection data and will likewise keep well away from sloppily mounted specimens.

Instead of sending specimens to an ant specialist, one may consider visiting a major ant collection to try one's own hand at identification. Proper preparation for such a visit should include preparation of and familiarity with a reference collection of dry-mounted specimens in one's collection. Make a list of priorities based on the most common ants and chose the 20–50 most common morphospecies in the research program. Take at least three pins of each morphospecies. Be sure to bring along a few extra pins of each species (properly labeled) that can be left for the specialist's ant collection.

Identifications are most easily carried out with the ant specialist guiding one through the key characteristics of each species. If the specialist does not have much time, one can also compare one's specimens to those in the specialist's collection. Once a verified identification has been made, another label should be added to the pin, with the species name, the name of the person who identified the specimen, and the date it was identified. If one compares one's specimens with the type specimen (the specimen used for originally naming the species), a colored label noting "Compared with type, [your name, date]" should be added. Specimens compared to a type specimen will be the most important future reference specimens in one's growing collection.

In looking for reference publications, it might be best to prioritize by first choosing keys to

general or regional faunas, then revisions of keys, then faunal lists, and finally the original species descriptions (Chapter 12). Growing knowledge of the literature will familiarize one with the specialists in a particular group, with whom one might begin a fruitful relationship (Chapters 5 and 12, and the social insects Web site).

## Building up a Morphospecies Reference Collection

Specimens of the identified morphospecies from each sample should be mounted and labeled in order to build up a reference collection, as discussed previously. This will permit comparison of the species collected from various sites and samples. Simply relying on an index card or fiche with some characters or illustrations as the only reference to a particular morphospecies will not do, since the difficulties that arise as new species are identified are best solved by keeping mounted samples of each morphospecies for comparison. The arrangement of a reference collection should best suit the needs of the particular project. In some cases it may be preferable to maintain reference collections separated by study site or project, thus reducing the amount of morphological variability owing to sampling from widely separated populations and facilitating comparisons.

Regardless of how collections are separated or joined, each one should have the ants arranged at least according to subfamily, genus, and species. The simplest and least complicated arrangement is alphabetical order starting with each subfamily, then putting all of its genera into alphabetical order, and then putting the species within each genus into alphabetical order. When only morphospecies are known, they are placed after the determined species of that genus and deposited in numerical order. This strategy permits easy retrieval and depositing of specimens by nonspecialists.

Eventually some groups of ants may grow in size to the point that trying to match so many morphospecies will become quite difficult. At this point one can attempt to divide each genus into groups of species that are similar in one or more diagnostic characters.

A fiche system can be grouped into nested sets of fiches: a set that guides to the subgroups of one genus and a set that guides to the species belonging to each subgroup. Alternatively a computerized database can be implemented, functioning as an electronic key.

## Building up a Proper Ant Collection

The development of proper taxonomic collections is beyond the scope of this book—and probably also beyond the interest and capacity of individual researchers and projects. The challenges are huge, especially in guaranteeing long-term funding and stable conditions for maintenance and curation beyond the individual researcher's working life.

However, the fundamental link between research collections and the results of inventory and biodiversity assessment studies lies in the deposition of voucher specimens in major ant collections, and in the integration of research collections at the end of a project or a scientist's career.

Though it is tempting to maintain an ever-growing ant collection, a few points should be considered before proceeding with such a strategy. The fact that one's investment, in both time and money, grows along with the collection has already been mentioned. With time, and a greater number of successful projects, more and more species in the collection will be described. In well-studied areas, a collection will therefore come to include few new species, whereas in such largely unstudied areas as Madagascar, up to 95% of all the species might be new to science. Following the laws of most countries, at

least the primary type specimens must be deposited in the country of origin.

Holotypes and, to a lesser degree, paratypes (specimens used to describe the species) play a key role in taxonomy, as they are the final authoritative reference for any species name. Thus they should be well preserved, accessible to scientists at any time, and, if possible, available for loan. It is a wonderful feeling to discover a new species, but housing the type specimens for it is a great responsibility.

It is therefore strongly recommended that the researcher establish a strong relationship with one or more of the major ant collections in his or her region (Chapter 12) before undertaking an ant diversity study. There are benefits to both partners: researchers gain access to new specimens, often in large numbers, which are often very rare in major collections and thus of high value to the collections. Researchers who deposit voucher specimens actively contribute to the growth of these institutions. Major collections usually have staff capable of identifying specimens for outside researchers, and they are often able to exchange specimens for other species of the region, or closely related species.

## Specimen Storage

Since only a sample of ants will be mounted, an additional group of specimens in alcohol will accompany the collection of mounted specimens. Specimens in alcohol should preferably be kept in an area separate from the dry collection.

Field numbers should readily be visible in alcohol vials and on mounted specimens so that the retrieval of a particular sample is relatively easy. Specimens may be ordered according to the field number for each sample, since each is distinct and permits the grouping of ants from the same area. The alcohol collection can also complement the dry collection when more specimens must be consulted for identification or

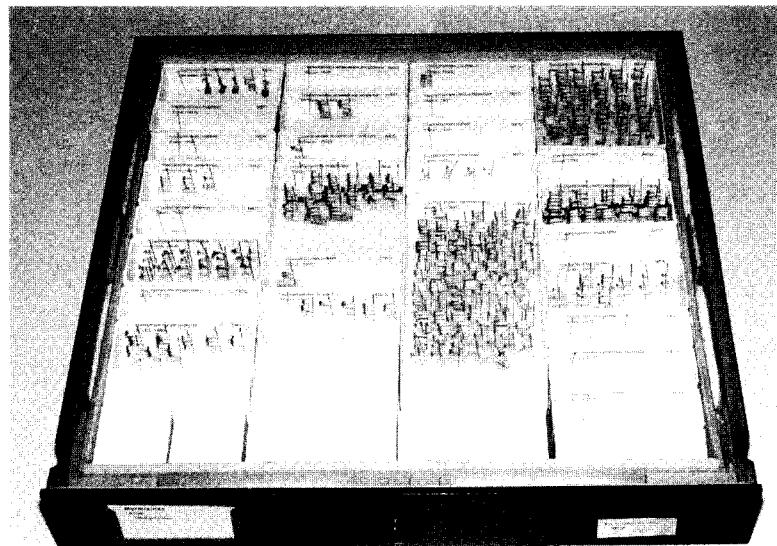


Figure 11.3. Mounted ants arranged in unit trays in a collection drawer. Photo by Ted Schultz.

new analysis, or if specimens are to be deposited in other collections.

The alcohol used for storing ants is ethanol at a concentration of 90% (the concentration should never drop below 70%; keep in mind that adding specimens will lower the concentration). Cheaper alcohol such as isopropyl alcohol will adversely affect the specimens. DNA analysis normally works best with specimens preserved in an ethanol concentration that is as high as possible. Thus preserving specimens in originally 95% pure ethanol is best. However, too high a concentration often stiffens specimens, making it more difficult to mount them properly later.

Vials for reliable long-term storage may be hard to come by in some countries. Vials designed for other purposes (e.g., centrifuge tubes) can be used, although the quality of the glass and lids is generally lower. Stoppers may deteriorate with time and need replacing. Cotton should never be used with specimens as the fibers entangle them, making observation difficult.

An alcohol collection should be checked at least once a year. If vials are almost empty, do not just add new ethanol. Rinse the vials several times with ethanol and then add new ethanol. The risk of desiccation of individual vials can be reduced by storing the vials in a larger jar that is also filled with ethanol. In case of complete desiccation of specimens, do not move them unnecessarily. Open the vial and put it into a wet chamber. This is normally made of a tightly closed box that has sterilized quartz sand on the bottom, which is kept humid with distilled water to which fungicide has been added. The specimens are kept there overnight; afterwards they are relaxed and perfect for handling. Two other techniques can also be used: either soak the specimens in hot water for few minutes or soak them in a solution of ammonia for a few hours.

Dry specimens should be stored in drawers that are as airtight as possible. If drawers are to be made locally, be very precise about the measurements in order to preserve uniformity in the

storage system and allow drawers to fit interchangeably into all storage cabinets. Unit trays are very strongly recommended, as they allow the movement of ants around in trays without moving individual pins (Fig. 11.3). Unit trays are commonly made out of cardboard with a foam bottom. They come in various sizes and are generally arranged together so that they add up to the size of the collection trays. Drawers and unit trays can be easily custom made or purchased from an entomological supply company (see Appendix 3).

The bottom of unit trays was originally made out of pressed peat, but today polyethylene or other foam is used. It is important that the foam not retain pin holes but be self-healing after pins are removed. This detail can save quite some time over the long term.

Pins should be of stainless steel, especially when they are to be used in humid tropical areas. Size 3 pins are preferred by many workers, as they are sturdy enough not to bend when replaced, although this is to some extent a question of taste and availability.

Finally, dry collections are best kept in rooms with climate control to protect against excessive heat, cold, and humidity. Excessive heat in combination with humidity makes specimens prime targets for fungi, insects, and mites; common pests include museum beetles (*Anthrenus* spp.), silverfish, and house lice. Damage can be prevented by adding some insecticide (e.g., camphor) to each box and especially by checking the collection at regular intervals, perhaps two or three times a year. Infested boxes should be taken away to be treated, making the process less of a health risk for workers in the laboratory, if the collection is housed in the same rooms as the laboratory.

## Specimen Shipping

To ship specimens to specialists or collections, use sturdy cardboard boxes padded with Styro-

foam chips to a thickness of at least 12 cm around the enclosed insect box. Use wide sticky tape to seal off all possible entrances to the box and cut off access by unwanted visitors, such as other insects, during the trip. If possible ship by an express courier (e.g., Federal Express, UPS, DHL), and do not forget the proper labels, including the return address. All appropriate customs forms should accompany international shipments to avoid delays at customs.

Vials containing specimens should be tightly bound together and sealed in a plastic bag to prevent spillage of ethanol if a vial should break or leak. Dry-mounted ant specimens should be firmly pinned in unit trays that are then secured within a cardboard box. Unit trays should be braced so that they do not move around in the box. Pin the ants as far down into the tray bottoms as possible with the help of a pair of strong forceps. If labels or triangles with specimens mounted on them are loose, either use thicker pins (size 3 pins usually prevent this problem) or (and for larger specimens this should be done as well) brace the specimen with a pin on each side of the cardboard triangle just in front of the specimen, so that it cannot rotate and thus fall off or destroy neighboring specimens.

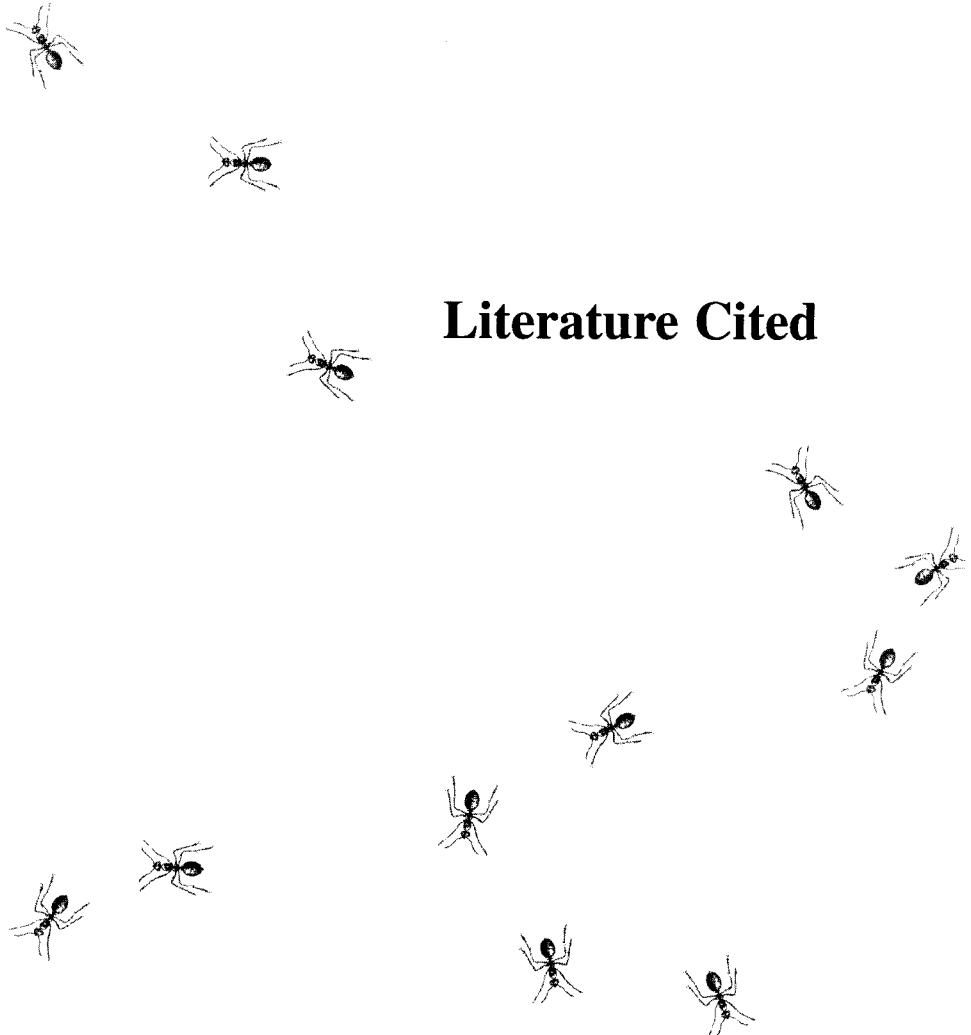
## Databases

The advent of personal computers, laser printers, and the Internet has made building a collection and managing its related data much simpler than in decades past. Once a new record has been entered into a database, it is easy to print labels, to search for specimens according to a particular locality or research question, to add images or drawings, to print distribution maps, or even to perform the daily bookkeeping of the collection. Maintaining an updated database also facilitates data analysis and allows one to check on questionable identifications or data easily.

Databases are available in many different formats. Depending on the size of the project, a simple spreadsheet program such as Excel may be sufficient. Such a program allows for the input, sorting, and export of data and the printing of labels. More advanced database programs normally require extra effort to learn and customize. BIOTA and BioLink are two that have been specifically developed for the purpose of specimen and species data handling and

might well serve the needs of most of the readers of this volume.

With a little patience and practice, the art of preparing and identifying and specimens can be mastered by anyone. A well-labeled reference collection serves as a solid baseline for ant studies and allows for comparisons to other sites and studies. By following the steps outlined in this chapter, the researcher can sort, mount, and identify ants relatively easily and rapidly.



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