Handbook on

Field Sampling Methods and Identification of Ants

(Family: Formicidae)

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Hand-book on Field Sampling and Laboratory Identification of Ants (Family: Formicidae), Third edition (First published as a book)

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CONTENTS

Introduction

Ants can be seen almost everywhere in indoors and outdoors and they may found colonies among the clothes, papers, decaying matter, in soil, on trees and shrubs and in many other microhabitats. Foraging worker ants may crawl long distances away from the nest in search of the food. Therefore, it is not easy to establish a single method for sampling ant species in a known area. A combination of methods can be applied to determine the species composition and relative abundance of ant species to match the objectives of the study.

Field sampling methods for ground-dwelling and ground foraging ants

Methods of sampling may vary according to the purpose of the study. Some methods are only suitable for the collection of ants in specific habitats. Myrmecologists all over the world collect ants by the following methods:

(1) Hand collection of worker ants with a pair of fine forceps (Plate 1)

Tips of the forceps are wetted with ethanol and the ants are easily collected by the forceps in to a small glass vial filled with 70% or 85% ethanol. This method is suitable for small to large sized worker ants and any other caste or life stage but special care should be taken to avoid damages to the body parts. This method alone is recommended for taxonomic purpose.

(2) Hand collection of workers with a fine-pointed (No. 0) paint brush (Plate 2)

A fine-pointed paint brush wetted with ethanol is used to pick life stages of ants instead of a pair of forceps. Small worker ants can easily be picked up with this method.

(3) Baited trapping (Plate 3)

Baits are types of food preferred by ants; honey, sugar, peanut butter, ground anchovies, powdered cheese, etc. can be used as baits for foraging worker ants. One or more baits can be used in combination if necessary. Food is usually added to a piece of square-shaped (2 cm × 2 cm) material such as gauze or a graph paper but small polythene bags or plastic cups can also be used for this purpose. Baits can be placed on the ground, among shrubs and on trees, and underground in pitfall traps or in plastic tubes (see (4) below). The bait is left for a period of time (e. g. one hour) necessary for the objective of the study; next, the worker ants attended to each bait are collected into a vial filled with 70% ethanol or the platform of the bait and all ants attended are directly collected into a labelled polythene bag or a plastic bottle. Mouth of the bag or the bottle should be closed properly with appropriate data labels to prevent the escaping of ants. All worker ants in each polythene bag or plastic bottle or 70 % ethanol should be sorted and transferred into a vial filled with 85% ethanol with an appropriate data labels, in the laboratory.

This method is commonly used to estimate the frequency of occurrence, species composition and species richness of the ground foraging worker ants in a selected region. This method is not suitable for the estimation of abundance of ant species.

(4) Pitfall trapping (Plate 4)

Pitfall traps are glass or plastic containers embedded in similar-sized pits made in the ground. A killing agent such as 70 % ethanol and a bait can be added to each container (Plate 4). After the period of time necessary for the objective of the study, ants fallen and trapped in the containers are collected into glass

vials filled with 70% - 85% ethanol. The collection in the containers may consist of debris, soil, other organisms and the ants. Therefore, special care is necessary when sorting ants collected in the pitfall traps.

Many soil-inhabiting cryptic ants can be collected with underground traps. According to Prof. Yamane, trapped ants are generally covered with soil particles and other debris; cleaning and identification of the specimens is time-consuming. Taxonomic specialists often refuse the identification of specimens collected with pitfall traps because they have to waste a lot of time; the specimens are not useful for taxonomic purpose.

Plate 1 A pair of fine forceps suitable for handling ants

Plate 2 A fine-pointed paint brush suitable for picking ants

Plate 3 A honey bait of a piece of gauze and a drop of honey

Plate 4 A pitfall trap

(5) Sampling ants or ant nests using a quadrat

A quadrat of a suitable size (Plate 5) is placed randomly in an open area and the ants present within the quadrat are collected into glass vials filled with 85% ethanol. A flexible quadrat (Plate 6) made up of a cord and four pegs fixed at the four corners in one meter or half a meter distance is useful for sampling ants in regions with the trees and shrubs such as forests.

This method is usually used to estimate the percentage abundance of worker ants and species composition of ground-dwelling and ground-foraging worker ants in a known area. Counting of all ants belonging to the same species within a quadrat area is a difficult task. Each ant sample should be labelled with an identification code.

This method can be applied to count the nests and estimate nest density of each ant species in a known area. This is done by throwing the quadrat and recording

the number of ant nests in each quadrat. Several workers from each nest should be collected and preserved in 70 % - 85 % ethanol for laboratory identification.

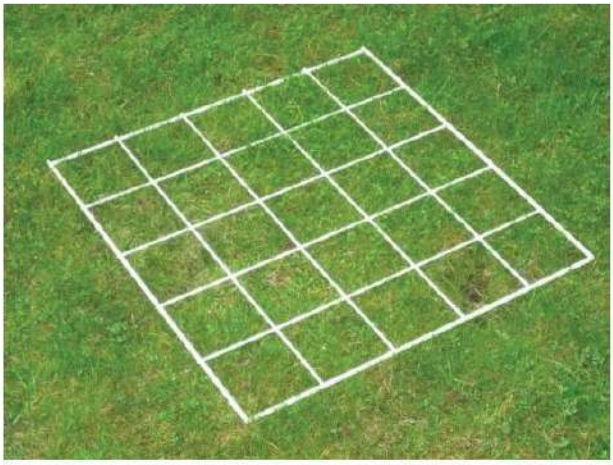

Plate 5 showing a quadrat

Plate 6 A flexible quadrat for regions with trees

(6) Sampling methods for leaf-litter associated ants

Litter sifters and Winkler sacks are commonly used.

(i) Hand-held litter sifter (Plate 7)

This litter sifter consists of a hand sieve or specially made rectangular mesh and a white tray of a suitable size convenient for handling. Leaf litter in a known area (e.g. 5 cm × 5 cm) or a volume is collected and added to the sieve (= mesh) while keeping the white tray underneath during the sifting. Ants associated with the leaf litter are separated by sifting leaf litter for a known time period (time unit sampling); all ants fallen to the white tray are collected using a pair of fine forceps or a paint brush into a bottle filled with 85% ethanol. This method of sampling is appropriate for ecological studies as well as for taxonomic purpose (Prof. Yamane, personal communication).

(ii) Winkler bag (Plate 8)

A Winkler bag consists of a metal, box-like frame that supports a covering made up of a canvas or cotton cloth. One or more smaller sacks of 4 mm mesh could be suspended inside a Winkler bag but a mini-Winkler bag (Plate 8) containing single smaller sack is preferred for the convenience. A receptacle, a plastic bag or a bottle, is attached to the bottom end of mini-Winkler bag. Leaf litter from the field is placed within a mesh and a sack is made; it is placed inside the mini-Winkler bag facilitating the downward ant movement. The set up should be kept for 24 hours or more to collect the ants in the sample.

Prof. Yamane elaborated that when managing large amount of litter, litter should be sieved for some time before putting into the sack; by this preliminary treatment more condensed material excluding leaves and other large particles can be separated; the Winkler method does not need electricity that is a prerequisite for Berlese (Tullgren) apparatus, a frequently used method for extracting small soil animals; the method is very efficient to extract leaf litter and surface soil ants, and allows the collection of many rare ants for taxonomic purpose.

(7) Soil sifting for sampling soil ants

A known volume of soil (e.g. 5 cm x 5 cm x 2 cm (depth)) is collected with appropriate tools (Plate 9) and sifted through the above-mentioned sieve (mesh) while keeping a white tray underneath of the mesh; ants fallen to the tray are collected and preserved in 70 % - 85 % ethanol. Ants remaining in the soil can be collected by a pair of fine forceps or a paint brush.

Very rare ants such as *Leptanilla, Anillomyrma*, etc., which generally do not appear in the samples collected by other methods can be sampled by this method (Prof. Yamane, personal communication).

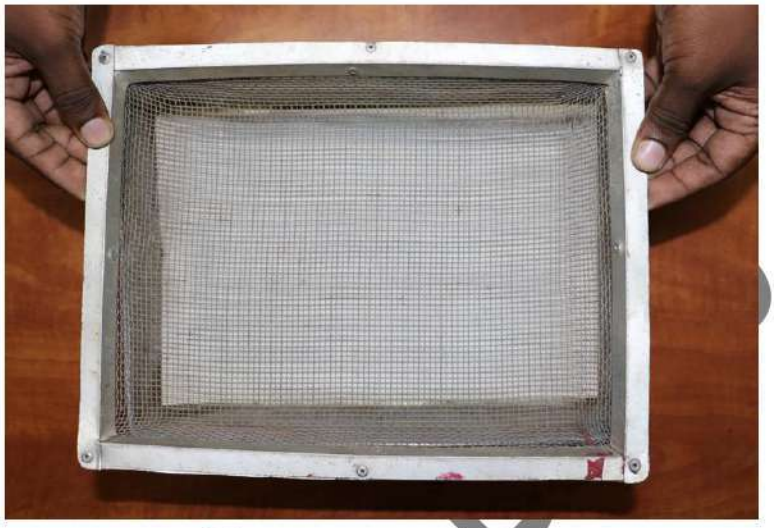

Plate 7 Hand-held litter sifter and the white tray for extracting ants in the leaf litter

Plate 8 Showing a mini-Winkler bag, which is used to collect ants in the leaf litter

Plate 9 Tools required for soil sifting

Plate 10 Sampling ants along a transect

(8) Intensive sampling of ants using simultaneous multiple methods

Worker ants are collected by two or more methods described previously to collect all ant species in a known area. For example, the ants seen in the leaf litter of a quadrat are, first collected manually and then, the leaf litter is subjected to Winkler extraction. Quadrat sampling, pitfall trapping and baiting can be conducted simultaneously to collect ants to match the objectives of the research. Two examples for simultaneous multiple methods are given below.

Example 1:

Along a 50 m or 100 m transect (Plate 10), soil sifting and pitfall trapping can be conducted at a preferred distance (e. g. 4 m or 5 m distance); suitable for the sampling of soil-dwelling ants and ground foraging ants.

Example 2:

Use soil sifting, litter sifting and hand-collection along a 50 m or 100 m transect and set pitfall traps throughout the selected area; suitable for the sampling of soil-dwelling ants and ground foraging ants.

Sampling methods for arboreal ants

According to the objectives of the survey, one or more methods can be used to sample worker ants on the trees. Collection of nests with all life stages is very useful for taxonomic purposes. Several methods are listed below.

(1) Sweeping of vegetation with insect nets

(2) Beating vegetation with a stick or shaking branches of trees vigorously to dislodge ants to white sheets or trays

(3) Hand collection of ants that inhabit vegetation

(4) Setting baited traps on tree branches (Plate 11)

(a)

(b)

Plate 11 (a) Beating/shaking the branches of tree while holding a sheet underneath (b) Baited trap set on a cashew tree

Prof. Seiki Yamane added the following paragraph:

Light traps, yellow pan trap (YP) and flight-interception trap (FIT) are employed to collect winged ants. Males and queens of some ant genera, particularly, *Camponotus*, *Polyrhachis*, *Odontomachus* etc., are attracted to the light; the queen and male in copula are seen around light, and establishment of queen-male combination of particular species is possible. Males of the army ant genus *Aenictus*, for example, can be collected by setting a FIT around sunset for a short period. Queens and males engaging in daytime nuptial flights are often attracted to yellow pan traps with water and a drop of detergent.

Preservation of collected ants

Ethyl alcohol (= ethanol) is recommended for the preservation of collected ants. For short term storage, 70 % - 80 % ethanol is recommended. For long term storage for taxonomic purposes, 85 % ethanol is recommended.

Preservation in Absolute Grade (ca. 100 %) of ethanol is required for DNA analysis of ants. Prof. Yamane mentions that specimens kept in 70 % – 80 % ethanol can be used for DNA analyses if they are transferred to absolute ethanol within two years.

Short term preservation in 70 % ethanol followed by dry mounting (see next section) is recommended for the museum collection of ants.

Preservation in Absolute ethanol (100%) or freezing at -20°C is recommended for molecular analysis of ants.

Taxonomically Important Morphological Features of Worker Ants

Morphological features that are important in identification of ants are shown in Figs. 1-3. Body of a worker ant is divisible in to four clearly separated regions, namely, the head, petiole, post-petiole and the gaster (Fig. 3).

Head:

The head (Fig. 1) usually bears a pair of compound eyes (but these are absent in some ants), a pair of antennae, ocelli and the mouthparts, a pair of mandibles and maxillae, a labrum (usually reduced), a labium and the hypopharynx. The antennae consist of the scape, an elongate basal segment and distal funiculus (flagellum) which may be of 3-11 smaller segments, depending on the ant genus or species. These segments may be filiform (Fig. 1(c)) or the apical 1- 4 may form a club (Fig. 1(a) & (b)). The scape has the ball-like articulatory bulb at its base (condylar bulb) followed by a short constriction (or "neck") and the scape (first antennal segment) distally. The condylar bulb articulates with the head in the antennal socket (antennal insertion), a foramen located behind the clypeus and this foramen is encircled by a narrow annular sclerite, the torulus. Also, the antennal socket may be overhung or concealed by the frontal lobe (Fig. 1(c)). In some ants, when each antenna is folded back, it is kept in a groove (= depression) running above or below the eyes on each side of the head and this groove is called the antennal scrobe (Fig. 3); antennal scrobe may only accommodate the scape. Clypeus is the sclerite at the anterior margin of the head in full-face view of many ants (but a projection of the labrum may be visible anteriorly in some ants) and has a taxonomic importance. Usually, the clypeus (Fig. 1(a) – (c))

consists of a median body and a pair of lateral carinae (longitudinal carinae) but various specializations can be seen among different ants. Among the mouthparts, the mandibles (Fig. 1(d) – (g)) of worker ants vary in shape, length and dentition and are very important in ant taxonomy. Each mandible has an inner margin (masticatory margin); it is usually armed with teeth, denticles or both (Fig. 1(d)). The basal margin of the mandible lies close to the articulation to the head capsule and these two margins may meet at the basal angle or tooth or through a narrow or broad curvature of the mandible. The external margin of each mandible forms its outer border in full–face view and may be straight, sinuate or convex (Fig. 1(e), (f) and (g)). Mandibles of the ants vary in shape and may be triangular or sub–triangular (Fig. 1(d) & (e)), elongate triangular (Fig. 1(g)) or linear (= the blade is long and narrow: apical and external margins are approximately parallel or taper towards the apex: the whole blade may be straight or curved) (Fig. 1(f)).

In ventral view of the head, maxillae are situated just above the two mandibles and the palps are articulated to the maxillae antero-laterally. The segmented sensory palps of the maxillae are known as maxillary palps (Fig. 1(c)) and each palp has several segments although the number of segments varies in the different ant groups. Short maxillary palps of some ants are not easily visible under a low power microscope. Maxillary palps are rarely absent in some ants. The labium is articulated to the head capsule ventrally in the central position of the buccal cavity. A pair of sensory palps arise antero-laterally on the labium and are known as labial palps (Fig. 1(d)). Short labial palps of some ants may not be easily visible under a low power microscope. Palp Formula (PF) is a standardized way of indicating the number of segments in the maxillary and

labial palps. The number of segments in the maxillary palp followed by that of the labial palp is mentioned as PF of a worker ant.

Example: PF = 3, 2 indicates that the maxillary palp has three segments and the labial palp has two segments.

The transverse posterior margin of the head in full-face view is termed as the 'occipital margin' by Bolton (1994) and is of taxonomic importance.

Alitrunk (mesosoma)

The alitrunk consists of pronotum, mesonotum, metanotum and the propodeum, the tergite of the first abdominal segment (Fig. 3). In some ants, the pronotum is fused with the mesonotum and there is no visible suture between the two segments. In others, promesonotal suture between the two segments are clearly visible. The metanotum may be present on the dorsum, reduced or obliterated. The mesonotum and propodeum are often separated by the metanotal groove (Fig. 3). The propodeum may be unarmed (Fig. 3) or armed with spiny projections.

The legs are articulated to each thoracic segment and each leg consists of a basal coxa, a small trochanter, a long femur, a tibia and a five-segmented tarsus that terminates in a pair of claws (Fig. 2(a)). A socketed spur is, usually, located at the apex of each tibia (Fig. 3). The fore-legs bear a single pectinate tibial spur whereas meso- and metathoracic tibiae may each bear one to two spurs or the latter two may be without spurs. When present, the spurs may be simple, pectinate or barbed. Pretarsal claws at the apex of the legs are also of taxonomic importance and usually, the inner curvature of each claw is a simple smooth

surface (Fig. 2(b)) but in some taxa, (1) one or more pre-apical teeth may be present along the inner curvature (Fig. 2(c)) or, (2) the claw may be pectinate.

Waist

Waist is a single segment (second abdominal segment) or two (second and third abdominal segments) separated segments that occur between the alitrunk and gaster (Fig. 3).

The single waist segment is the petiole and, if two waist segments are present, those are known as petiole and the post-petiole. Morphologically, petiole is the second abdominal segment and the post-petiole is the third abdominal segment. Generally, the petiole appears as a node or a scale of different shape and size but in some ants it may be reduced and concealed by the gaster. The petiole has the second abdominal spiracle and usually it consists of a distinct tergite and a sternite although these two segments may be fused in some ants.

The helcium (Fig. 3) is also taxonomically important in some ants and this consists of presclerites of the third abdominal segment, which form a complex articulation within the posterior foramen of the petiole. In some ants, helcium is only partly visible.

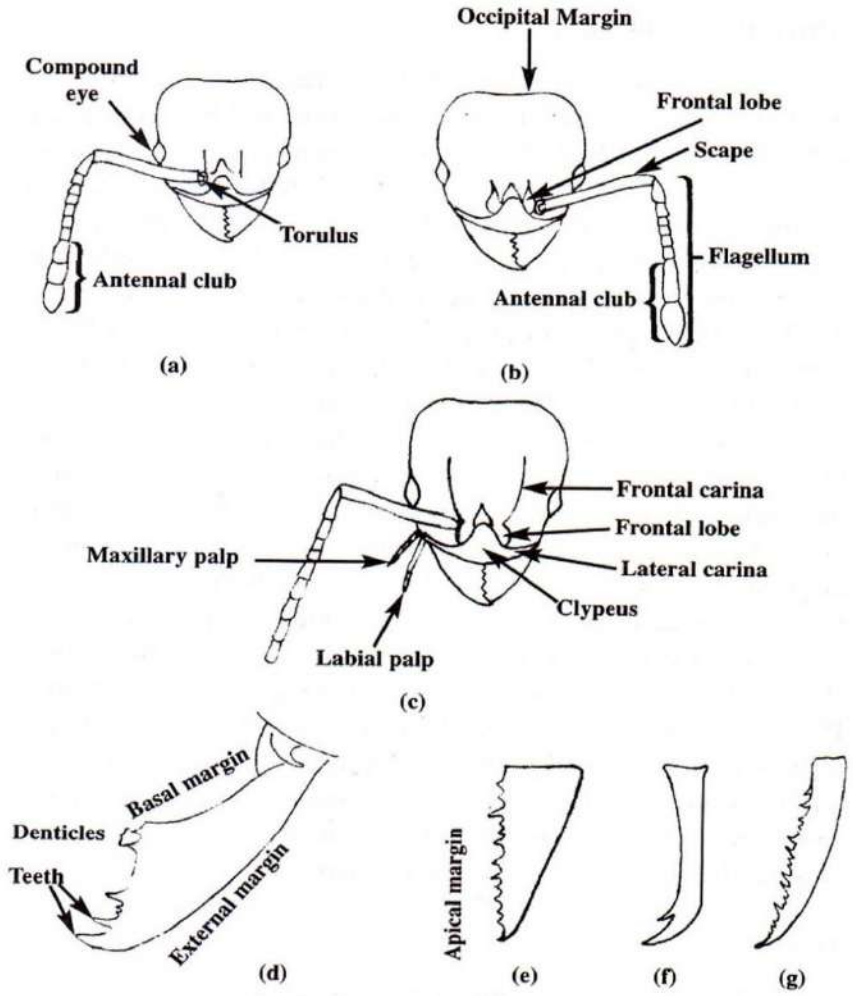

Figure 1 Morphological features of the head region and associated appendages of a worker ant (Dias, 2002)

(a), (b), (c) – Frontal view of the head

(d) – Enlarged triangular mandible showing detailed structure

(e) – Triangular or subtriangular mandible

(f) – Linear mandible

(g) – Elongate triangular mandible

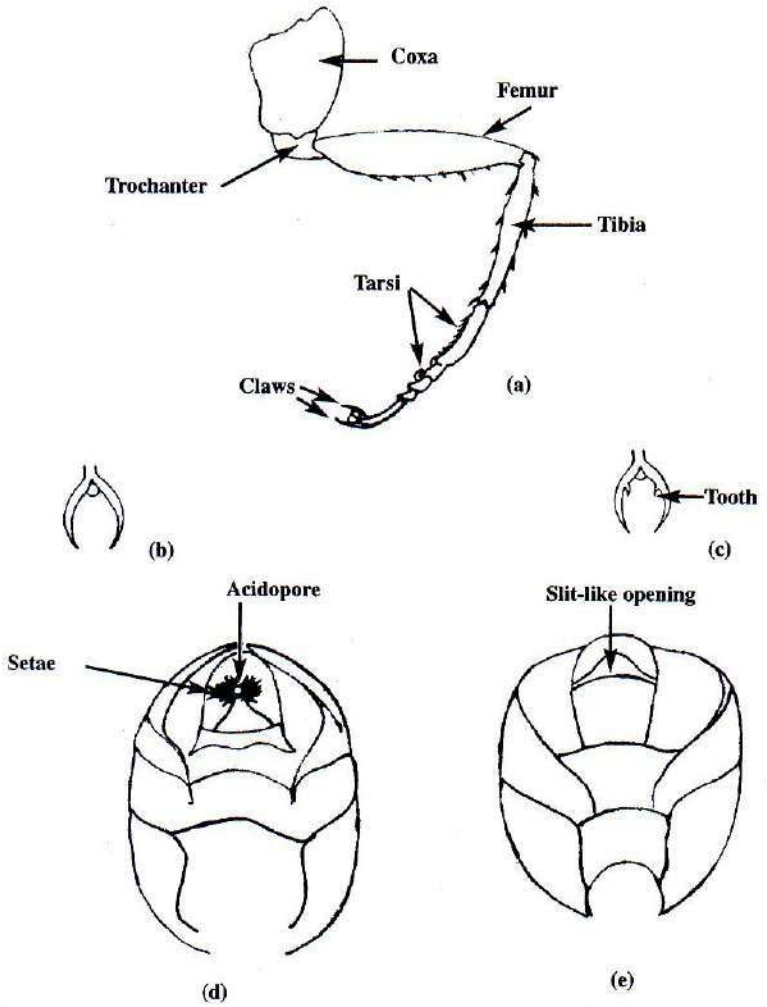

Figure 2 Taxonomically important morphological features seen on a leg and gaster of a worker ant (Dias, 2002)

(a) A leg

(b) Simple claws

(c) Claws with an additional tooth

(d) Ventral side of gaster showing the acidopore

(e) Ventral side of gaster showing the absence of an acidopore

Gaster

The gaster of ants consists of 3-7 or 4-7 abdominal segments depending on whether the waist is formed of a single segment or two segments, respectively. Taxonomically important features of this body region are (a) the presence or absence of a constriction between the gastral segments 1 and 2 (Fig. 3), (b) the presence or absence of projections on pygidium (the last visible gastral tergite), (c) the structure of hypopygium (the last visible sternite), (d) the presence or absence of a sting (Fig. 3), and (e) the presence (Fig. 2(d)) or absence of a circular acidopore ventrally (Fig. 2(e)).

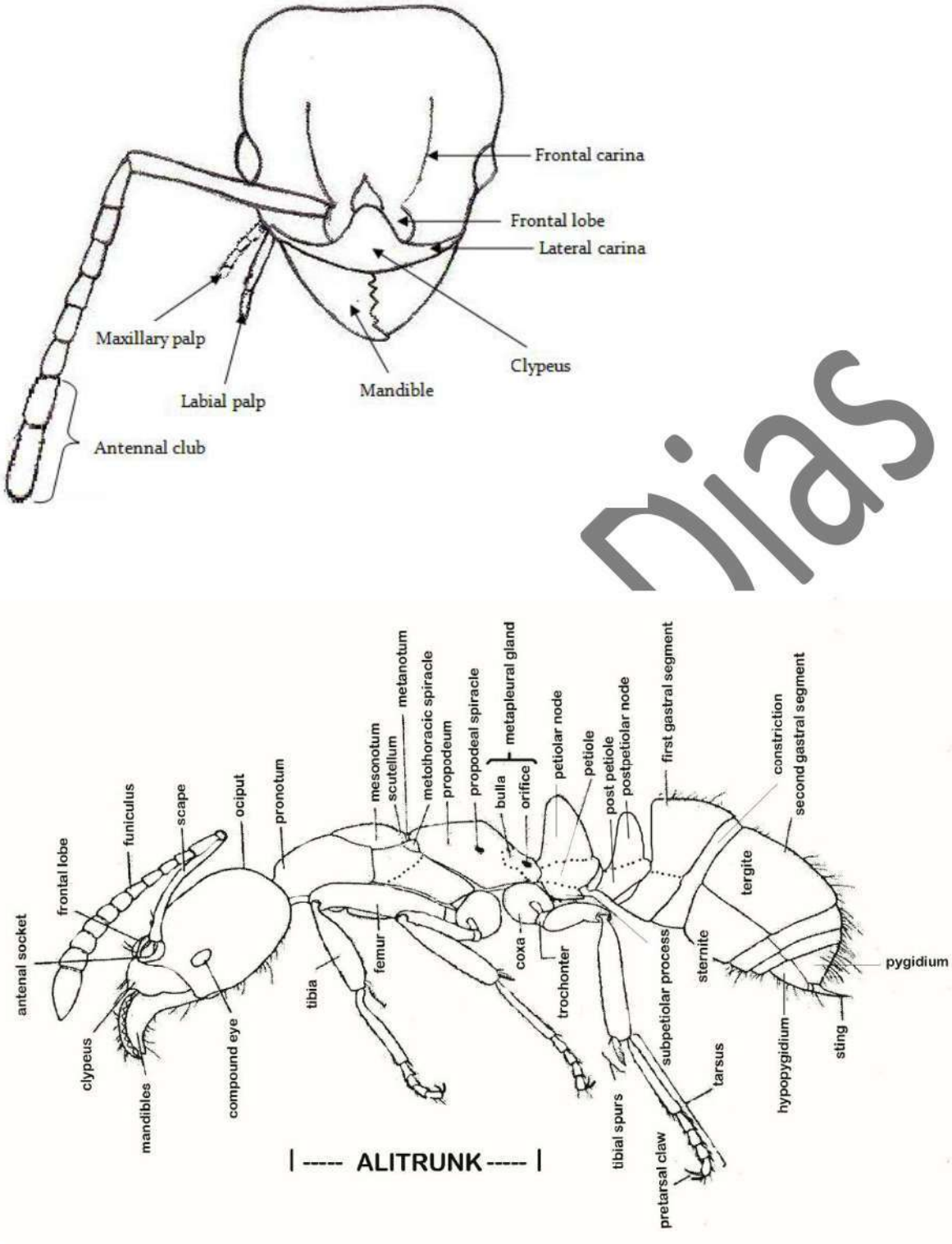

Figure 3 Taxonomically important morphological features in a frontal view (top) and a lateral view of a worker ant (Dias, 2014)

Laboratory Methods for the Identification of Worker Ants

(a) Quick observations

A worker ant preserved in ethanol could be directly observed under a low power microscope after allowing the specimen to dry well on a filter paper. Wet specimens do not clearly show some morphological features.

Remember that specimens without data labels are often mixed with other ants. This causes a serious problem to the quality (confidence) of samples. Identification of specimens should principally be done after they are mounted on points (small triangle pieces of hard paper mounted on a pin) with data labels (Prof. Yamane, personal communication).

(b) Observations for taxonomic purpose

Preparation of a dry mount (Plate 13) of a worker ant is recommended. Dry the specimen by keeping it on a filter paper (blotting paper) for few minutes; observe under a low power stereo-microscope (Plate 14) at suitable magnifications and mount the ant as instructed in the next section. Preparation of such a dry mount (see below) will also allow easy handling of the specimen.

(c) Studying ant images for the identification

A digital camera (which can focus well with appropriate zoom levels) attached to a Low Power Stereomicroscope (several brands available) is recommended for photographing ants (Plate 14). The digital camera projects the image to the computer in an enlarged view and taxonomically important features of very small ants can also be seen easily. Two such images of ants are provided in Plate 20. Although expensive a scanning electron microscope is more suitable for getting images of ants for taxonomic purpose.

Plate 13 Dry mounted worker ants (images from Prof. Seiki Yamane)

Plate 14 Low Power Stereomicroscope with an attached digital camera

How to prepare a dry mount of a worker ant

Materials required for the preparation of a double mount are as follows:

- Card triangles ("points"): Plate 15
- Insect pins (No.3 & 5 preferred): Plate 16
- Step block :Plate 17 (a) and 17 (b)
- Water soluble and quickly drying glue
- A pair of fine forceps (Plate 1) and a paint brush (Plate 2)
- Insect display box (or any box of convenient size): Plate 18

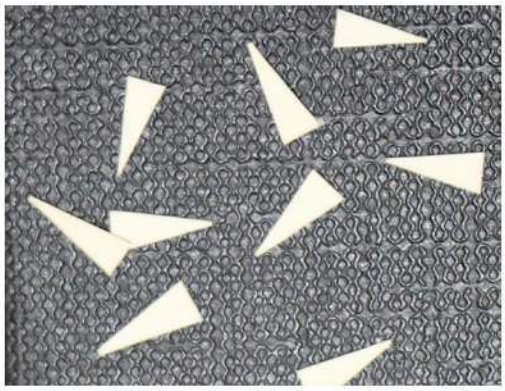

Plate 15 Showing the card triangles or "points"

Plate 16 Insect pins (of stainless steel)

(a)

(b)

Plate 17 (a) Wooden step block and (b) metal step block

Plate 18 Insect storage or display box

Follow the steps given below to prepare a dry mount of a worker ant.

(a) Place a card triangle on the step block.

(b) Pin (usually a No. 3 insect pin is used) through the centre base of the card triangle (Plate 13) and move the triangle up carefully by fine forceps. The distance between the pin head and the point is usually about $\frac{1}{3}^{rd}$ of the total length of the pin.

(c) Pick a worker ant in ethanol and place it on a filter paper or blotting paper and let it become dry as much as possible. Next, evert the mandibles by gently pressing down the head with two insect pins (Please note that this should be done while observing the specimen under a low power microscope if the worker ant is small).

(d) Apply a minimum amount of glue with an insect pin on the pointed tip of the card triangle fixed to the step block and place the ant transversely on its dorsal side while holding it with a fine forceps or a fine paint brush. Head of the ant must be on the right hand side when the tapering end of the card triangle is directed away from the mounter. The coxae of middle and hind legs should only be in contact with glue. DO NOT EMBED THE ANT in the glue.

Note: Prepare several mounts of similar or different types of workers.

(e) A label or several labels with collection data (usually of rectangular-shape) must also accompany each ant mounted in this manner (Plate 13).

(i) The exact locality (if available latitude/longitude and altitude are useful)

(ii) Date of collection

(iii) Habitat, nest site, method of sampling

(iv) Name of the collector

(v) Level of identification and name of the determiner

(vi) Colony code when it was collected from a colony (for example, SD16-SL-152)

(f) For the determination of the taxonomic level, the mounted ant should be observed under a Low Power Stereo-microscope at suitable magnifications. This dry mount should finally be pinned in an insect storage or display box.

(g) Drying of the mounted ants is necessary for the long term preservation. Insect storage or display box containing dry mounts can be kept at $35C^0$ in an oven for 3 - 4 days.

Several wet-preserved specimens of the same species are necessary for the study of antennae, mandibles, maxillary palps and the labial palps. If the ant is small (2 mm or less in body length), above appendages should be dissected out carefully under a Low Power Stereo-microscope, kept on a microscopic slide with a drop of glycerin and observed under a High-power microscope at higher magnifications. This is usually required for counting the number of segments in the antenna of small ants.

Identification of worker ants to the possible taxonomic level

Place a dry mounted ant under the Low Power Stereo-microscope and observe all taxonomically important features of the specimen. Taxonomic keys (if available) for subfamilies, genera and species should be followed for the identification.

The first valuable guide to the ant genera of the world was "An Identification Guide to the Ant Genera of the World" by Mr. Barry Bolton published in 1994 (Plate 19); revisions to his guide were added by publishing "Synopsis and classification of Formicidae" in 2003. Many revisions in the subfamily, generic and species levels of ants since 2003 have been published; a current

researcher on ants should search for all such references to identify ants, which is a really difficult task.

Several websites on the ant diversity are:

Bolton's Catalogue of the ants of the world, http://www.antweb.org,

http://www.antbase.org,

http://www.antwiki.org,

http://www.kln.ac.lk/science/antDB/ (Ants of Sri Lanka only)

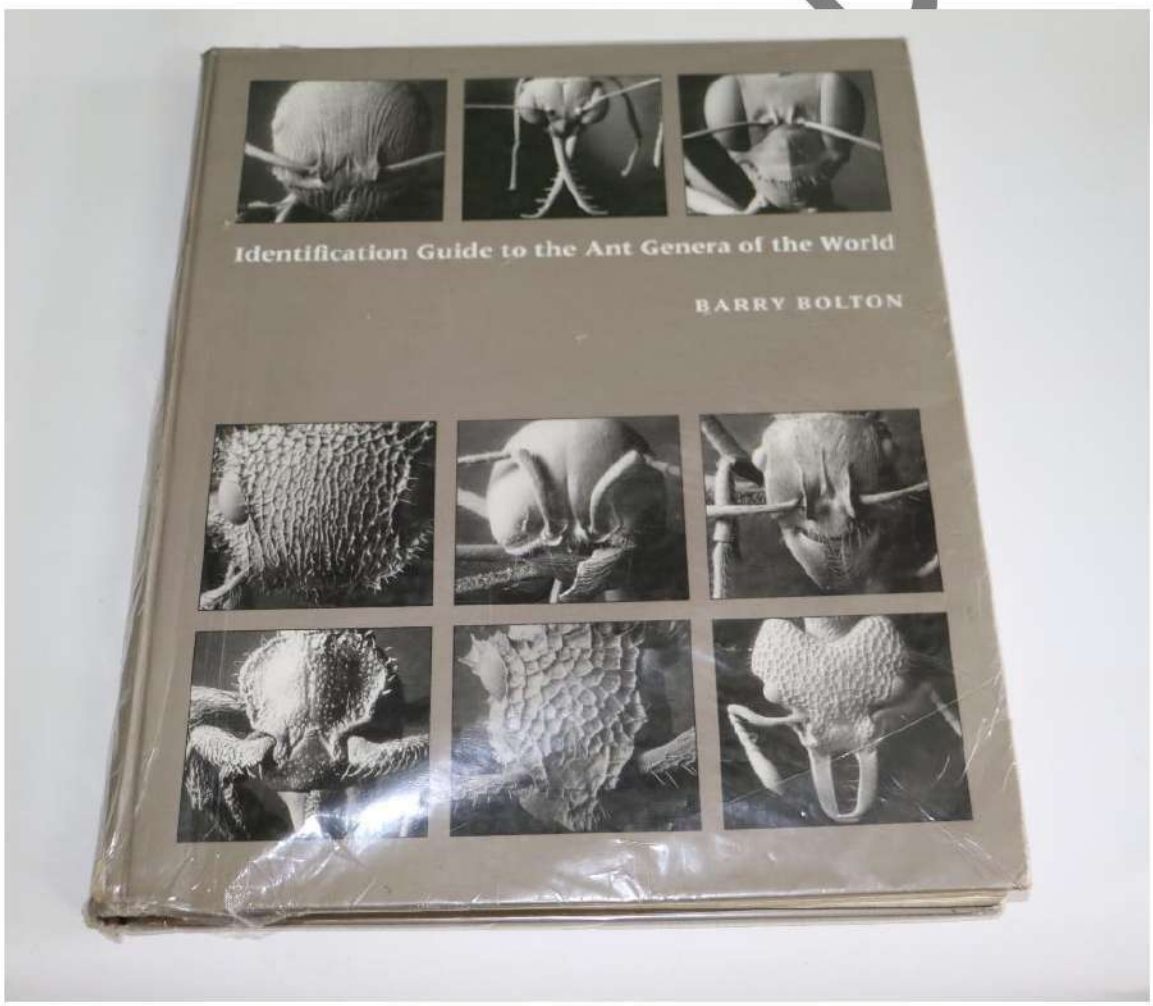

Plate 19 Front cover of Bolton's Identification guide published in 1994

Plate 20 Images of ants taken with a digital camera annexed to a stereo-microscope

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DATA ANALYSIS

This training course is not intended to provide hands-on experience in the data analysis. The annexed published research papers will provide you examples for the data analysis and publishing of your findings.